```
FILE 'HOME' ENTERED AT 10:09:24 ON 24 FEB 2009

=> index bioscience
FILE 'DRUGMONOG' ACCESS NOT AUTHORIZED
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                       0.22       0.22

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE,
       AQUASCI, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CAPLUS,
       CEABA-VTB, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DISSABS, DRUGB,
       DRUGMONOG2, DRUGU, EMBAL, EMBASE, ...' ENTERED AT 10:10:04 ON 24 FEB 2009

68 FILES IN THE FILE LIST IN STNINDEX

Enter SET DETAIL ON to see search term postings or to view
search error messages that display as 0* with SET DETAIL OFF.

=> s (individual or single) (s) (metaboli## or (oxygen (2a) consum?))
        756   FILE ADISCTI
        673   FILE ADISINSIGHT
        480   FILE ADISNEWS
       4360   FILE AGRICOLA
        724   FILE ANABSTR
        107   FILE ANTE
        395   FILE AQUALINE
       2949   FILE AQUASCI
       3285   FILE BIOENG
      14335   FILE BIOSIS
       2949   FILE BIOTECHABS
       2949   FILE BIOTECHDS
       7841   FILE BIOTECHNO
      12535   FILE CABA
      14858   FILE CAPLUS
        290   FILE CEABA-VTB
         34   FILE CIN
        110   FILE CONFSCI
         65   FILE CROPB
        467   FILE CROPU
        135   FILE DDFB
       4766   FILE DDFU
     425999   FILE DGENE
  23 FILES SEARCHED...
       2865   FILE DISSABS
        135   FILE DRUGB
       9394   FILE DRUGU
        146   FILE EMBAL
      12602   FILE EMBASE
      45566   FILE ESBIOBASE
          2   FILE FOMAD
        925   FILE FROSTI
       1367   FILE FSTA
       1091   FILE GENBANK
        490   FILE HEALSAFE
       2180   FILE IFIPAT
         72   FILE IMSDRUGNEWS
        176   FILE IMSRESEARCH
        146   FILE KOSMET
      13749   FILE LIFESCI
      13401   FILE MEDLINE
        884   FILE NTIS
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         22   FILE NUTRACEUT
  45 FILES SEARCHED...
       1003   FILE OCEAN
      20384   FILE PASCAL
         20   FILE PHAR
         32   FILE PHARMAML
        280   FILE PHIN
       2031   FILE PROMT
         45   FILE PROUSDDR
          5   FILE RDISCLOSURE
      10967   FILE SCISEARCH
          1   FILE SYNTHLINE
       8866   FILE TOXCENTER
       2740   FILE USGENE
  59 FILES SEARCHED...
      26890   FILE USPATFULL
        292   FILE USPATOLD
       4980   FILE USPAT2
          7   FILE VETB
        331   FILE VETU
        776   FILE WATER
       2110   FILE WPIDS
         14   FILE WPIFV
       2110   FILE WPINDEX

  63 FILES HAVE ONE OR MORE ANSWERS,   68 FILES SEARCHED IN STNINDEX

L1   QUE (INDIVIDUAL OR SINGLE) (S) (METABOLI## OR (OXYGEN (2A) CONSUM?))

=> s L1 (s) (microplate or microwell or chip or well)
         82   FILE ADISCTI
        235   FILE ADISINSIGHT
        127   FILE ADISNEWS
        696   FILE AGRICOLA
        107   FILE ANABSTR
         23   FILE ANTE
         53   FILE AQUALINE
        451   FILE AQUASCI
        599   FILE BIOENG
        431   FILE BIOSIS
        434   FILE BIOTECHABS
        434   FILE BIOTECHDS
       1274   FILE BIOTECHNO
       1769   FILE CABA
        417   FILE CAPLUS
         42   FILE CEABA-VTB
          2   FILE CIN
  19 FILES SEARCHED...
         22   FILE CROPU
        423   FILE DDFU
       2304   FILE DGENE
  23 FILES SEARCHED...
        417   FILE DISSABS
       1008   FILE DRUGU
          9   FILE EMBAL
        429   FILE EMBASE
       4158   FILE ESBIOBASE
         47   FILE FROSTI
        146   FILE FSTA
         39   FILE GENBANK
         80   FILE HEALSAFE
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        220   FILE IFIPAT
         21   FILE IMSDRUGNEWS
        106   FILE IMSRESEARCH
         50   FILE KOSMET
       2210   FILE LIFESCI
        454   FILE MEDLINE
  43 FILES SEARCHED...
        154   FILE NTIS
          4   FILE NUTRACEUT
        146   FILE OCEAN
       3532   FILE PASCAL
          8   FILE PHARMAML
         32   FILE PHIN
        304   FILE PROMT
          7   FILE PROUSDDR
          2   FILE RDISCLOSURE
        375   FILE SCISEARCH
        256   FILE TOXCENTER
  58 FILES SEARCHED...
        277   FILE USGENE
       5300   FILE USPATFULL
         64   FILE USPATOLD
        858   FILE USPAT2
         19   FILE VETU
  64 FILES SEARCHED...
        149   FILE WATER
        143   FILE WPIDS
        143   FILE WPINDEX

  54 FILES HAVE ONE OR MORE ANSWERS,   68 FILES SEARCHED IN STNINDEX

L2   QUE L1 (S) (MICROPLATE OR MICROWELL OR CHIP OR WELL)

=> s L2 (s) (cell or embryo# or blastocyst# or oocyte#)
          2   FILE ADISCTI
         99   FILE ADISINSIGHT
         39   FILE ADISNEWS
        149   FILE AGRICOLA
         13   FILE ANABSTR
          4   FILE ANTE
          9   FILE AQUALINE
        102   FILE AQUASCI
        214   FILE BIOENG
         42   FILE BIOSIS
        301   FILE BIOTECHABS
        301   FILE BIOTECHDS
  12 FILES SEARCHED...
        538   FILE BIOTECHNO
        343   FILE CABA
         54   FILE CAPLUS
         14   FILE CEABA-VTB
  17 FILES SEARCHED...
         33   FILE DDFU
        850   FILE DGENE
  23 FILES SEARCHED...
        131   FILE DISSABS
        114   FILE DRUGU
         34   FILE EMBASE
       1139   FILE ESBIOBASE
  30 FILES SEARCHED...
          6   FILE FROSTI
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         24   FILE FSTA
         35   FILE GENBANK
          9   FILE HEALSAFE
        153   FILE IFIPAT
          1   FILE IMSDRUGNEWS
         37   FILE IMSRESEARCH
         22   FILE KOSMET
        713   FILE LIFESCI
         42   FILE MEDLINE
         36   FILE NTIS
  44 FILES SEARCHED...
         26   FILE OCEAN
        796   FILE PASCAL
  49 FILES SEARCHED...
          1   FILE PHARMAML
          6   FILE PHIN
         64   FILE PROMT
          3   FILE PROUSDDR
          1   FILE RDISCLOSURE
         52   FILE SCISEARCH
         23   FILE TOXCENTER
  59 FILES SEARCHED...
       1870   FILE USPATFULL
         17   FILE USPATOLD
        287   FILE USPAT2
          2   FILE VETU
         17   FILE WATER
         63   FILE WPIDS
         63   FILE WPINDEX

  49 FILES HAVE ONE OR MORE ANSWERS,   68 FILES SEARCHED IN STNINDEX

L3   QUE L2 (S) (CELL OR EMBRYO# OR BLASTOCYST# OR OOCYTE#)

=> s L3 (s) gradient
          7   FILE AGRICOLA
          1   FILE ANABSTR
          1   FILE AQUALINE
          3   FILE AQUASCI
          6   FILE BIOENG
          7   FILE BIOTECHABS
          7   FILE BIOTECHDS
  12 FILES SEARCHED...
         10   FILE BIOTECHNO
         11   FILE CABA
          1   FILE CAPLUS
  16 FILES SEARCHED...
  23 FILES SEARCHED...
          4   FILE DISSABS
          1   FILE DRUGU
         19   FILE ESBIOBASE
  30 FILES SEARCHED...
          9   FILE GENBANK
         17   FILE IFIPAT
          3   FILE KOSMET
         10   FILE LIFESCI
  43 FILES SEARCHED...
          2   FILE OCEAN
         12   FILE PASCAL
  47 FILES SEARCHED...
  59 FILES SEARCHED...
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         21   FILE USPATFULL
          5   FILE USPAT2
          1   FILE WPIDS
          1   FILE WPINDEX

  23 FILES HAVE ONE OR MORE ANSWERS,   68 FILES SEARCHED IN STNINDEX

L4   QUE L3 (S) GRADIENT

=> d rank
F1          21   USPATFULL
F2          19   ESBIOBASE
F3          17   IFIPAT
F4          12   PASCAL
F5          11   CABA
F6          10   BIOTECHNO
F7          10   LIFESCI
F8           9   GENBANK
F9           7   AGRICOLA
F10          7   BIOTECHABS
F11          7   BIOTECHDS
F12          6   BIOENG
F13          5   USPAT2
F14          4   DISSABS
F15          3   AQUASCI
F16          3   KOSMET
F17          2   OCEAN
F18          1   ANABSTR
F19          1   AQUALINE
F20          1   CAPLUS
F21          1   DRUGU
F22          1   WPIDS
F23          1   WPINDEX


=> fil f2, f3, f5-f7, f9-f15
COST IN U.S. DOLLARS                                  SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                        21.76      21.98

FILE 'ESBIOBASE' ENTERED AT 10:29:08 ON 24 FEB 2009
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FILE 'IFIPAT' ENTERED AT 10:29:08 ON 24 FEB 2009
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FILE 'CABA' ENTERED AT 10:29:08 ON 24 FEB 2009
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FILE 'BIOTECHNO' ENTERED AT 10:29:08 ON 24 FEB 2009
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FILE 'LIFESCI' ENTERED AT 10:29:08 ON 24 FEB 2009
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FILE 'AGRICOLA' ENTERED AT 10:29:08 ON 24 FEB 2009

FILE 'BIOTECHABS' ACCESS NOT AUTHORIZED

FILE 'BIOTECHDS' ENTERED AT 10:29:08 ON 24 FEB 2009
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FILE 'BIOENG' ENTERED AT 10:29:08 ON 24 FEB 2009

FILE 'USPAT2' ENTERED AT 10:29:08 ON 24 FEB 2009

FILE 'DISSABS' ENTERED AT 10:29:08 ON 24 FEB 2009

FILE 'AQUASCI' ENTERED AT 10:29:08 ON 24 FEB 2009

=> s L4
1 FILES SEARCHED...
5 FILES SEARCHED...
L5 99 L4

=> dup rem L5
PROCESSING COMPLETED FOR L5
L6 66 DUP REM L5 (33 DUPLICATES REMOVED)

=> s L6 and py<2004
3 FILES SEARCHED...
10 FILES SEARCHED...
L7 33 L6 AND PY<2004

=> d L7 ibib abs 1-33

L7 ANSWER 1 OF 33 Elsevier BIOBASE COPYRIGHT 2009 Elsevier Science B.V. on STN
ACCESSION NUMBER: 2003097206 ESBIOBASE <<LOGINID::20090224>>
TITLE: Hepatotoxicity and mechanism of action of haloalkanes: Carbon tetrachloride as a toxicological model
AUTHOR: Weber L.W.D.; Boll M.; Stampfl A.
CORPORATE SOURCE: L.W.D. Weber, Institute of Toxicology, GSF-Natl. Res. Ctr. Environ./Hlth., P.O. Box 1129, D-85758 Neuherberg, Germany.
E-mail: lwdweber@yahoo.com
SOURCE: Critical Reviews in Toxicology, (2003), 33/2 (105-136), 292 reference(s)
CODEN: CRTXB2 ISSN: 1040-8444
DOCUMENT TYPE: Journal; General Review
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The use of many halogenated alkanes such as carbon tetrachloride (CCl.sub.4), chloroform (CHCl.sub.3) or iodoform (CHI.sub.3), has been banned or severely restricted because of their distinct toxicity. Yet CCl.sub.4 continues to provide an important service today as a model substance to elucidate the mechanisms of action of hepatotoxic effects such as fatty degeneration, fibrosis, hepatocellular death, and carcinogenicity. In a matter of dose, exposure time, presence of potentiating agents, or age of the affected organism, regeneration can take place and lead to full recovery from liver damage. CCl.sub.4 is activated by cytochrome (CYP)2E1, CYP2B1 or CYP2B2, and possibly CYP3A, to form the trichloromethyl radical, CCl.sub.3*. This radical can bind to cellular molecules (nucleic acid, protein, lipid), impairing crucial cellular processes such as lipid metabolism, with the potential outcome of fatty degeneration (steatosis). Adduct formation between

CCl.sub.3* and DNA is thought to function as initiator of hepatic cancer. This radical can also react with oxygen to form the trichloromethylperoxy radical CCl.sub.3OO*, a highly reactive species. CCl.sub.3OO* initiates the chain reaction of lipid peroxidation, which attacks and destroys polyunsaturated fatty acids, in particular those associated with phospholipids. This affects the permeabilities of mitochondrial, endoplasmic reticulum, and plasma membranes, resulting in the loss of cellular calcium sequestration and homeostasis, which can contribute heavily to subsequent cell damage. Among the degradation products of fatty acids are reactive aldehydes, especially 4-hydroxynonenal, which bind easily to functional groups of proteins and inhibit important enzyme activities. CCl.sub.4 intoxication also leads to hypomethylation of cellular components; in the case of RNA the outcome is thought to be inhibition of protein synthesis, in the case of phospholipids it plays a role in the inhibition of lipoprotein secretion. None of these processes per se is considered the ultimate cause of CCl.sub.4-induced cell death; it is by cooperation that they achieve a fatal outcome, provided the toxicant acts in a high single dose, or over longer periods of time at low doses. At the molecular level, CCl.sub.4 activates tumor necrosis factor (TNF)α, nitric oxide (NO), and transforming growth factors (TGF)-α and -β in the cell, processes that appear to direct the cell primarily toward (self-)destruction or fibrosis. TNFα pushes toward apoptosis, whereas the TGFs appear to direct toward fibrosis. Interleukin (IL)-6, although induced by TNFα, has a clearly antiapoptotic effect, and IL-10 also counteracts TNFα action. Thus, both interleukins have the potential to initiate recovery of the CCl.sub.4-damaged hepatocyte. Several of the above-mentioned toxication processes can be specifically interrupted with the use of antioxidants and mitogens, respectively, by restoring cellular methylation, or by preserving calcium sequestration. Chemicals that induce cytochromes that metabolize CCl.sub.4, or delay tissue regeneration when co-administered with CCl.sub.4, will potentiate its toxicity thoroughly, while appropriate CYP450 inhibitors will alleviate much of the toxicity. Oxygen partial pressure can also direct the course of CCl.sub.4 hepatotoxicity. Pressures between 5 and 35 mmHg favor lipid peroxidation, whereas absence of oxygen, as well as a partial pressure above 100 mmHg, both prevent lipid peroxidation entirely. Consequently, the location of CCl.sub.4-induced damage mirrors the oxygen gradient across the liver lobule. Mixed halogenated methanes and ethanes, found as so-called disinfection byproducts at low concentration in drinking water, elicit symptoms of toxicity very similar to carbon tetrachloride, including carcinogenicity.

L7 ANSWER 2 OF 33 Elsevier BIOBASE COPYRIGHT 2009 Elsevier Science B.V. on STN

ACCESSION NUMBER: 2003069886 ESBIOBASE <<LOGINID::20090224>>

TITLE: abcb1ab P-glycoprotein is involved in the uptake of citalopram and trimipramine into the brain of mice

AUTHOR: Uhr M.; Grauer M.T.

CORPORATE SOURCE: M. Uhr, Max Planck Institute of Psychiatry, Kraepelinstrasse 10, D-80804 Munich, Germany. E-mail: uhr@mpipsykl.mpg.de

SOURCE: Journal of Psychiatric Research, (2003), 37/3 (179-185), 38 reference(s)
CODEN: JPYRA3 ISSN: 0022-3956

DOCUMENT TYPE: Journal; Article

COUNTRY: United Kingdom

LANGUAGE: English

SUMMARY LANGUAGE: English

AB The phenomenon of a heterogeneous response to the same drug in different

patients is well-known. An important reason is that, even at equal concentrations, the bioavailability of a drug depends on the interaction of the drug with the blood-brain barrier (BBB). In part, this is due to the drug-transporting P-glycoprotein (P-gp), a product of the multiple drug resistance gene (ABCB1), which can transport drugs against a concentration gradient across the BBB back into the plasma and thereby reduce the bioavailability in the brain. In the present study, we have examined the uptake of the antidepressants citalopram and trimipramine into the brain of abcb1ab knockout mice compared with controls. One hour after s.c. injection of the drugs, concentrations of the two drugs and of the metabolite d-trimipramine in brain, spleen, kidney, liver and plasma were measured with HPLC. Significantly higher brain concentrations in knockout mice, showing that these drugs are substrates of P-gp and that the presence of P-gp reduces the effective bioavailability of these substances in the brain. The results of our study contradict an earlier report that citalopram is not actively transported from endothelial cells. These results were derived from an in vitro study, showing that due to the complexity of the BBB-drug interaction, it is difficult to transfer results from in vitro studies to the in vivo situation. We hypothesize that inter-individual differences in the activity of the ABCB1 gene can account in part for the great variation in clinical response to antidepressants in psychiatric patients, even at comparable plasma levels.

L7   ANSWER 3 OF 33 Elsevier BIOBASE

ACCESSION NUMBER: 2003014828   ESBIOBASE <<LOGINID::20090224>>

TITLE: Tobacco transformants with strongly decreased expression of pyrophosphate:fructose-6-phosphate expression in the base of their young growing leaves contain much higher levels of fructose-2,6-bisphosphate but no major changes in fluxes

AUTHOR: Nielsen T.H.; Stitt M.
CORPORATE SOURCE: T.H. Nielsen, Department of Plant Biology, Roy. Vet./Agricultural University, Thorvaldsensvej 40, 1871, Frederiksberg C, Denmark.
E-mail: thni@kvl.dk

SOURCE: Planta, (2001), 214/1 (106-116), 23 reference(s)
CODEN: PLANAB ISSN: 0032-0935
DOCUMENT TYPE: Journal; Article
COUNTRY: Germany, Federal Republic of
LANGUAGE: English
SUMMARY LANGUAGE: English

AB   The role of pyrophosphate:fructose-6-phosphate 1-phosphotransferase (PFP) in developing leaves was studied using wild-type tobacco (Nicotiana tabacum L.) and transformants with decreased expression of PFP. (i) The leaf base, which is the youngest and most actively growing area of the leaf, had 2.5-fold higher PFP activity than the leaf tip. T3 transformants, with a 56-95% decrease in PFP activity in the leaf base and an 87-97% decrease in PFP activity at the leaf tip, were obtained by selfing and re-selfing individuals from two independent transformant lines. (ii) Other enzyme activities also showed a gradient from the leaf base to the leaf tip. There was a decrease in PFK and an increase in fructose-6-phosphate,2-kinase and plastidic fructose-1, 6-bisphosphatase, whereas cytosolic fructose-1,6-bisphosphatase activity was constant. None of these gradients was altered in the transformants. (iii) Fructose-2,6-bisphosphate (Fru2,6bisP) levels were similar at the base

and tip of wild-type leaves in the dark. Illumination lead to a decrease in Fru2,6bisP at the leaf tip and an increase in Fru2,6bisP at the leaf base. Compared to wild-type plants, transformants with decreased expression of PFP had up to 2-fold higher Fru2,6bisP at the leaf tip in the dark, similar levels at the leaf tip in the light, 15-fold higher levels at the leaf base in the dark, and up to 4-fold higher levels at the leaf base in the light. (iv) To investigate metabolic fluxes, leaf discs were supplied with .sup.1.sup.4CO.sub.2 in the light or [.sup.1.sup.4C]glucose in the light or the dark. Discs from the leaf tip had higher rates of photosynthesis than discs from the leaf base, whereas the rate of glucose uptake and metabolism was similar in both tissues. Significantly less label was incorporated into neutral sugars, and more into anionic compounds, cell wall and protein, and amino acids in discs from the leaf base. Metabolism of .sup.1.sup.4CO.sub.2 and [.sup.1.sup.4C]glucose in transformants with low PFP was similar to that in wild-type plants, except that synthesis of neutral sugars from .sup.4CO.sub.2 was slightly reduced in discs from the base of the leaf. (v) These results reveal that the role of PFP in the growing cells in the base of the leaf differs from that in mature leaf tissue. The increase in Fru2,6bisP in the light and the high activity of PFP relative to cytosolic fructose-1,6-bisphosphatase in the base of the leaf implicate PFP in the synthesis of sucrose in the light, as well as in glycolysis. The large increase in Fru2,6bisP at the base of the leaf of transformants implies that PFP plays a more important role in metabolism at the leaf base than in mature leaf tissue. Nevertheless, there were no major changes in carbon fluxes, or leaf or plant growth in transformants with below 10% of the wild-type PFP activity at the leaf base, implying that large changes in expression can be compensated by changes in Fru2,6-bisP, even in growing tissues.

L7 ANSWER 4 OF 33 Elsevier BIOBASE COPYRIGHT 2009 Elsevier Science B.V. on STN

ACCESSION NUMBER: 2002261060 ESBIOBASE <<LOGINID::20090224>>
TITLE: Ternary gradient elution markedly improves silver-ion high performance liquid chromatography of unsaturated sterols
AUTHOR: Shan H.; Wilson W.K.
CORPORATE SOURCE: W.K. Wilson, Department of Biochemistry, Rice University, 6100 Main Street, Houston, TX 77005-1892, United States.
E-mail: billw@rice.edu
SOURCE: Steroids, (2002), 67/11 (917-923), 24 reference(s)
CODEN: STEDAM ISSN: 0039-128X
PUBLISHER ITEM IDENT.: S0039128X02000569
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AB A wide variety of unsaturated sterols can accumulate in eukaryotic cells as a consequence of normal metabolism, genetic disorders, and actions of enzyme inhibitors. Resolving these sterol mixtures into individual components by conventional chromatographic methods is inefficient because unsaturated sterols differ little in polarity, hydrophobicity, and volatility. Although sterol mixtures are well-resolved by silver-ion high performance liquid chromatography (Ag.sup.+-HPLC), existing methods require derivatization to acetates for best results, and the isocratic mobile phases lead to long analysis times and low sensitivity for late-eluting sterols. We show that these problems can be overcome with ternary gradient elution using acetone, hexanes, and acetonitrile.

Separation of a mixture of 20 underivatized sterols gave dramatically shortened analysis times, with good peak shapes for both early- and late-eluting components. In a similar separation of blood sterols from a patient with Smith-Lemli-Opitz syndrome, the band for 7-dehydrocholesterol was much narrower than with isocratic elution. Column re-equilibration was rapid, and the separations could be monitored with ultraviolet spectroscopy at 210nm, which affords universal, non-destructive detection of unsaturated sterols. Also discussed are retention mechanisms and reproducibility of Ag.sup.+-HPLC separations. The overall results represent a major advance in chromatographic methods for resolving mixtures of unsaturated sterols differing in the number and position of olefinic bonds.

L7 ANSWER 5 OF 33 Elsevier BIOBASE COPYRIGHT 2009 Elsevier Science B.V. on STN

ACCESSION NUMBER: 2002249018 ESBIOBASE <<LOGINID::20090224>>

TITLE: Influence of pH conditions on metabolic regulations in serine alkaline protease production by Bacillus licheniformis

AUTHOR: Calik P.; Bilir E.; Calik G.; Ozdamar T.H.

CORPORATE SOURCE: T.H. Ozdamar, Dept. of Industrial Biotechnology, Biotechnology Research Center, Ankara University, Tandogan, 06100 Ankara, Turkey.
E-mail: ozdamar@science.ankara.edu.tr

SOURCE: Enzyme and Microbial Technology, (03 OCT 2002) , 31/5 (685-697), 17 reference(s)
CODEN: EMTED2 ISSN: 0141-0229

PUBLISHER ITEM IDENT.: S014102290200162X

DOCUMENT TYPE: Journal; Article

COUNTRY: United States

LANGUAGE: English

SUMMARY LANGUAGE: English

AB The effects of the controlled and uncontrolled pH conditions, as well as of the value of initial pH in the range of 7-7.5, on serine alkaline protease (SAP) production by Bacillus licheniformis were investigated on a defined medium with the single carbon source glucose in batch bioreactors. Besides uncontrolled pH operations, growth phase, production phase, and entire process control strategies were also applied. The concentrations of the product (SAP) and by-products, i.e. neutral protease, amylase, amino acids, and organic acids were determined in addition to SAP activities. For SAP production, uncontrolled pH operation was more favourable than the controlled pH operations; and pH.sub.0=7.25 was optimum for SAP production where maximum enzyme activity was obtained as 390Ucm.sup.-.sup.3, while, the highest cell concentration was obtained at pH.sub.0=7 in uncontrolled operation. Thereafter, by using the experimental data obtained at pH.sub.0=7, 7.25 and 7.5 uncontrolled pH operations, perturbation effects of pH on the intracellular flux distributions were investigated for the growth (0<t<20h) and SAP production (20<t<=43h) periods. In the growth phase the fluxes of the glycolysis pathway and the TCA cycle increased with the increase in pH.sub.0; further, due to the difference in proton electrochemical gradients generated, the lowest and the highest energies were produced at pH.sub.0=7 and 7.5 conditions, respectively. In the SAP production period, at pH.sub.0=7.5 and 7 the glycolysis pathway fluxes were, respectively, the lowest and the highest. However, the TCA cycle fluxes, amino acid synthesis fluxes and SAP synthesis flux were the highest at pH.sub.0=7.25 condition. The diversions in the pathways and certain metabolic reactions and potential strategies for improving SAP production are also discussed.

L7 ANSWER 6 OF 33 Elsevier BIOBASE COPYRIGHT 2009 Elsevier Science B.V. on STN

ACCESSION NUMBER: 2002082087 ESBIOBASE <<LOGINID::20090224>>

TITLE: Activity and diversity of sulfate-reducing bacteria in a petroleum hydrocarbon-contaminated aquifer

AUTHOR: Kleikemper J.; Schroth M.H.; Sigler W.V.; Schmucki M.; Bernasconi S.M.; Zeyer J.

CORPORATE SOURCE: J. Kleikemper, Inst. Terrestrial Ecol.-Soil Biol., Swiss Federal Inst. Technol. Zurich, CH-8952 Schlieren, Switzerland.
E-mail: kleikemper@ito.umnw.ethz.ch

SOURCE: Applied and Environmental Microbiology, (2002), 68/4 (1516-1523), 55 reference(s)
CODEN: AEMIDF ISSN: 0099-2240

DOCUMENT TYPE: Journal; Article

COUNTRY: United States

LANGUAGE: English

SUMMARY LANGUAGE: English

AB Microbial sulfate reduction is an important metabolic activity in petroleum hydrocarbon (PHC)-contaminated aquifers. We quantified carbon source-enhanced microbial SO.sub.4.sup.2.sup.- reduction in a PHC-contaminated aquifer by using single-well push-pull tests and related the consumption of sulfate and added carbon sources to the presence of certain genera of sulfate-reducing bacteria (SRB). We also used molecular methods to assess suspended SRB diversity. In four consecutive tests, we injected anoxic test solutions (1,000 liters) containing bromide as a conservative tracer, sulfate, and either propionate, butyrate, lactate, or acetate as reactants into an existing monitoring well. After an initial incubation period, 1,000 liters of test solution-groundwater mixture was extracted from the same well. Average total test duration was 71 h. We measured concentrations of bromide, sulfate, and carbon sources in native groundwater as well as in injection and extraction phase samples and characterized the SRB population by using fluorescence in situ hybridization (FISH) and denaturing gradient gel electrophoresis (DGGE). Enhanced sulfate reduction concomitant with carbon source degradation was observed in all tests. Computed first-order rate coefficients ranged from 0.19 to 0.32 day.sup.-.sup.1 for sulfate reduction and from 0.13 to 0.60 day.sup.-.sup.1 for carbon source degradation. Sulfur isotope fractionation in unconsumed sulfate indicated that sulfate reduction was microbially mediated. Enhancement of sulfate reduction due to carbon source additions in all tests and variability of rate coefficients suggested the presence of specific SRB genera and a high diversity of SRB. We confirmed this by using FISH and DGGE. A large fraction of suspended bacteria hybridized with SRB-targeting probes SRB385 plus SRB385-Db (11 to 24% of total cells). FISH results showed that the activity of these bacteria was enhanced by addition of sulfate and carbon sources during push-pull tests. However, DGGE profiles indicated that the bacterial community structure of the dominant species did not change during the tests. Thus, the combination of push-pull tests with molecular methods provided valuable insights into microbial processes, activities, and diversity in the sulfate-reducing zone of a PHC-contaminated aquifer.

L7 ANSWER 7 OF 33 Elsevier BIOBASE COPYRIGHT 2009 Elsevier Science B.V. on STN

ACCESSION NUMBER: 2000214653 ESBIOBASE <<LOGINID::20090224>>

TITLE: The structural properties of plant peroxisomes and their metabolic significance

AUTHOR: Reumann S.

CORPORATE SOURCE: S. Reumann, Albrecht-von-Haller, Institut fur Pflanzenwissenschaften, Abteilung fur Biochemie der Pflanze, Untere Karspule 2, D-37073 Gottingen, Germany.
E-mail: sreuman@gwdg.de

SOURCE: Biological Chemistry, (2000), 381/8 (639-648), 94 reference(s)
CODEN: BICHF3 ISSN: 1431-6730

DOCUMENT TYPE: Journal; General Review

COUNTRY: Germany, Federal Republic of

LANGUAGE: English

SUMMARY LANGUAGE: English

AB Plant peroxisomes can be isolated by Percoll density gradient centrifugation at high purity and metabolic competence as well as in relatively large quantities. According to biochemical and electrophysiological analyses, plant peroxisomes have recently been shown to differ from other cell organelles in essential structural properties. Unlike mitochondria or plastids, compartmentalization of plant peroxisomal metabolism is in major parts not caused by a boundary function of the membrane but is primarily due to the specific structure of the protein matrix. The enzymes of the photorespiratory C.sub.2 cycle of leaf peroxisomes are arranged as multienzyme complexes that allow efficient metabolic channelling with high flux rates and minimum leakage of reactive oxygen species from the organelle. Transfer of metabolites, such as carboxylates, proceeds across the peroxisomal membrane via a porin-like channel, which represents a relatively unspecific but highly efficient transport system. Because all variants of peroxisomes, which all contain only a single boundary membrane, are confronted with the task of transporting a large group of metabolites while preventing the escape of reactive intermediates, it is reasonable to speculate that the unique compartmentalization feature of leaf peroxisomes also applies to peroxisomes from fungi and mammals.

L7 ANSWER 8 OF 33 Elsevier BIOBASE COPYRIGHT 2009 Elsevier Science B.V. on STN

ACCESSION NUMBER: 2000126130 ESBIOBASE <<LOGINID::20090224>>

TITLE: Phenotypic variations in the gills of the symbiont-containing bivalve Lucinoma aequizonata

AUTHOR: Hentschel U.; Millikan D.S.; Arndt C.; Cary S.C.; Felbeck H.

CORPORATE SOURCE: U. Hentschel, Inst. fur Molek. Infektionsbiologie, Universitat Wurzburg, D-97070 Wurzburg, Germany.
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SOURCE: Marine Biology, (2000), 136/4 (633-643), 36 reference(s)
CODEN: MBIOAJ ISSN: 0025-3162

DOCUMENT TYPE: Journal; Article

COUNTRY: Germany, Federal Republic of

LANGUAGE: English

SUMMARY LANGUAGE: English

AB The marine bivalve Lucinoma aequizonata (Lucinidae) maintains a population of sulfide-oxidizing chemoautotrophic bacteria in its gill tissue. These are housed in large numbers intracellularly in specialized host cells, termed bacteriocytes. In a natural population of L. aequizonata, striking variations of the gill colors occur, ranging from yellow to grey, brown and black. The aim of the present study was to investigate how this phenomenon relates to the physiology and numbers of the symbiont population. Our results show that in aquarium-maintained animals, black gills contained fewer numbers of bacteria as well as lower concentrations of sulfur and total protein. Nitrate respiration

was stimulated by sulfide (but not by thiosulfate) 33-fold in homogenates of black gills and threefold in yellow gill homogenates. The total rates of sulfide-stimulated nitrate respiration were the same. Oxygen respiration could be measured in animals with yellow gills but not in animals with black gills. The cumulative data suggest that black-gilled clams maintained in the aquarium represent a starvation state. When collected from their natural habitat black gills contain the same number of bacteria as yellow gills. Also, no significant difference in glycogen concentrations of the host tissues was observed. Therefore, starvation is unlikely the cause of black gill color in a natural population. Alternative sources of nutrition to sulfur-based metabolism are discussed. Denaturing gradient gel electrophoresis (DGGE) performed on the different gill tissues, as well as on isolated symbionts, resulted in a single gill symbiont amplification product, the sequence of which is identical to published data. These findings provide molecular evidence that one dominant phylotype is present in the morphologically different gill tissues. Nevertheless, the presence of other phylotypes cannot formally be excluded. The implications of this study are that the gill of L. aequizonata is a highly dynamic organ which lends itself to more detailed studies regarding the molecular and cellular processes underlying nutrient transfer, regulation of bacterial numbers and host-symbiont communication.

L7 ANSWER 9 OF 33 Elsevier BIOBASE COPYRIGHT 2009 Elsevier Science B.V. on STN

ACCESSION NUMBER: 1999184767 ESBIOBASE <<LOGINID::20090224>>
TITLE: Membranes as possible pacemakers of metabolism
AUTHOR: Hulbert A.J.; Else P.L.
CORPORATE SOURCE: A.J. Hulbert.
E-mail: hulbert@uow.edu.au
SOURCE: Journal of Theoretical Biology, (07 AUG 1999), 199/3 (257-274), 98 reference(s)
CODEN: JTBIAP ISSN: 0022-5193
DOCUMENT TYPE: Journal; Article
COUNTRY: United Kingdom
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Basal metabolic rate (BMR) varies dramatically among vertebrate species, both (i) being several fold higher in the endothermic mammals and birds compared to the ectothermic reptiles, amphibians and fish, and (ii) being much greater, on a body mass basis, in small vertebrates compared to large vertebrates. These differences in whole animal BMR are also manifest at the cellular level with substantial contributions to basal metabolic activity from the maintenance of various trans-membrane gradients. The percentage contribution of various processes to basal metabolism is remarkably consistent between different vertebrates and when BMR varies, the components of metabolic activity vary in relative unison. Membrane composition also varies between vertebrates and the degree of polyunsaturation of membrane phospholipids is correlated with cellular metabolic activily. In general, the tissue phospholipids and thus membrane bilayers of endotherms are more polyunsaturated than those from similar-sized ectotherms. In mammals membrane polyunsaturation is allometrically related to body mass. We suggest that membranes can act as pacemakers for overall metabolic activity. We propose that such membrane polyunsaturation increases the molecular activity of many membrane-bound proteins and consequently some specific membrane leak-pump cycles and cellular metabolic activity. We hypothesize a possible mechanistic basis for this effect that is based on a greater transfer of energy during intermolecular collisions of membrane proteins with the

unsaturated two carbon units (C=C) of polyunsaturates compared to the single carbon units of saturated acyl chains, as well as the more even distribution of such units throughout the depth of the bilayer when membranes contain polyunsaturated acyl chains compared to monounsaturated ones. The proposed pacemaker role of differences in membrane bilayer composition is briefly discussed with respect to the brain (and sensory cells), evolution of mammalian endothermic metabolism, and its clinical implications for humans.

L7 ANSWER 10 OF 33 Elsevier BIOBASE on STN

ACCESSION NUMBER: 1999152073 ESBIOBASE <<LOGINID::20090224>>
TITLE: Kinetic aspects of drug disposition in the lungs
AUTHOR: Upton R.N.; Doolette D.J.
CORPORATE SOURCE: Dr. R.N. Upton, Dept. of Anaesthesia/Intensive Care, Royal Adelaide Hospital, University of Adelaide, Adelaide, SA 5005, Australia.
E-mail: rupton@health.adelaide.edu.au
SOURCE: Clinical and Experimental Pharmacology and Physiology, (1999), 26/5-6 (381-391), 80 reference(s)
CODEN: CEXPB0 ISSN: 0305-1870
DOCUMENT TYPE: Journal; General Review
COUNTRY: Australia
LANGUAGE: English
SUMMARY LANGUAGE: English

AB 1. The pharmacokinetic role of the lungs has been extensively studied using in vitro preparations, but this information has not been well integrated into many systemic pharmacokinetic models. 2. The lung is characterized by short diffusion distances, extremely high relative perfusion and heterogeneous cell types. Anionic and neutral lipophilic drugs have relatively small distribution volumes in the lungs due to their low lipid content. Cationic lipophilic drugs can accumulate in the lungs, probably due to trapping in mitochondria and lysosomes, forming very slowly eluting pools. 3. Drug metabolism in the lungs is possible, but not universal. The lung, generally, has a low activity for many of the metabolic enzymes found in the liver, although this activity is relatively more inducible. The resultant drug extraction would be 'enzyme limited', variable and flow dependent. 4. Double indicator studies of first-pass lung kinetics can characterize short- term distribution in the lungs, but not longer-term distribution or metabolism; the converse applies for studies of drug concentration gradients across the lungs. No single study or model has adequately defined the short- and long-term kinetics of drugs in the lungs. 5. Drug clearance in the lungs can contribute to an apparent total body clearance in excess of hepatic blood flow and cardiac output. The lung is a first pass filter for any drug administered on the venous side of the circulation and can act as a 'capacitor' that damps the first-pass concentration peak in the blood after intravenous bolus injection.

L7 ANSWER 11 OF 33 Elsevier BIOBASE on STN

ACCESSION NUMBER: 1997115817 ESBIOBASE <<LOGINID::20090224>>
TITLE: Three-dimensional imaging of rhodamine 123 fluorescence distribution in human melanoma cells by means of confocal laser scanning microscopy
AUTHOR: Porwol T.; Merten E.; Opitz N.; Acker H.
CORPORATE SOURCE: Prof. Dr. H. Acker, Max-Planck Inst. Mol. Physiologie, Rheinlanddamm 201, D-44139 Dortmund, Germany.
SOURCE: Acta Anatomica, (1996), 157/2 (116-125), 29 reference(s)

CODEN: ACATA5 ISSN: 0001-5180
DOCUMENT TYPE: Journal; Article
COUNTRY: Switzerland
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Three-dimensional (3D) imaging of intracellular rhodamine 123 fluorescence distribution was performed by means of confocal laser scanning microscopy (CLSM). Human IGR melanoma cells grown in monolayer or multicellular spheroid culture were studied for elucidating mitochondrial membrane potential characteristics, and cell and nucleus volume dimensions. Microspheres 6 μm in diameter loaded with rhodamine B were used to calibrate our instruments for performing 3D imaging of optical sections as obtained by CLSM. Accurate optical slicing is only possible taking into consideration the physical characteristics of the objectives used like chromatic and spherical aberrations, depth discrimination or cover slip correction and the temperature dependence of the immersion medium. While 3D imaging of optical slices can be carried out showing the original shape of the object being tested without physical distortion, 3D images of microspheres show well -reproducible structures of rhodamine B fluorescence. These can be explained by a superposition of two effects, namely scattering of the fluorescence light and a gradient of the electromagnetic field strength of the laser beam due to the shape of the object. 3D imaging of optical slices of IGR cells in monolayer or multicellular spheroid culture, which have been loaded with rhodamine 123, show the location of the dye predominantly within the cytoplasm of the cells with a remarkable heterogeneity of fluorescence intensity within and between single cells, indicating differences in the mitochondrial membrane potential and thus in the metabolic activity. Due to the heterogeneity of the cell shape the cell nucleus occupies between 4 and 14% of the total cell volume. These data reveal calibrated 3D imaging as a valuable noninvasive tool to visualize the heterogeneity of cell parameters under different cell culture conditions.

L7 ANSWER 12 OF 33 Elsevier BIOBASE COPYRIGHT 2009 Elsevier Science B.V. on STN
ACCESSION NUMBER: 1997108971 ESBIOBASE <<LOGINID::20090224>>
TITLE: Taurine reduction in anaerobic respiration of Bilophila wadsworthia RZATAU
AUTHOR: Laue H.; Denger K.; Cook A.M.
CORPORATE SOURCE: A.M. Cook, Facultat fur Biologie, Die Universitat, D-78434 Konstanz, Germany.
E-mail: Alasdair.Cook@uni-konstanz.de
SOURCE: Applied and Environmental Microbiology, (1997), 63/5 (2016-2021), 49 reference(s)
CODEN: AEMIDF ISSN: 0099-2240
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Organosulfonates are important natural and man-made compounds, but until recently (T. J. Lie, T. Pitta, E. R. Leadbetter, W. Godchaux III, and J. R. Leadbetter. Arch. Microbial. 166:204-210, 1996), they were not believed to be dissimilated under anoxic conditions. We also chose to test whether alkane- and arenesulfonates could serve as electron sinks in respiratory metabolism. We generated 60 anoxic enrichment cultures in mineral salts medium which included several potential electron donors and a single organic sulfonate as an electron sink, and we used material from anaerobic digestors in communal sewage works as inocula. None of the four aromatic sulfonates, the three

unsubstituted alkanesulfonates, or the N-sulfonate tested gave positive enrichment cultures requiring both the electron donor and electron sink for growth. Nine cultures utilizing the natural products taurine, cysteate, or isethionate were considered positive for growth, and all formed sulfide. Two clearly different pure cultures were examined. Putative Desulfovibrio sp. strain RZACYSA, with lactate as the electron donor, utilized sulfate, aminomethanesulfonate, taurine, isethionate, and cysteate, converting the latter to ammonia, acetate, and sulfide. Strain RZATAU was identified by 16S rDNA analysis as Bilophila wadsworthia. In the presence of, e.g., formate as the electron donor, it utilized, e.g., cysteate and isethionate and converted taurine quantitatively to cell material and products identified as ammonia, acetate, and sulfide. Sulfite and thiosulfate, but not sulfate, were utilized as electron sinks, as was nitrate, when lactate was provided as the electron donor and carbon source. A growth requirement for 1,4-naphthoquinone indicates a menaquinone electron carrier, and the presence of cytochrome e supports the presence of an electron transport chain. Pyruvate-dependent disappearance of taurine from cell extracts, as well as formation of alanine and release of ammonia and acetate, was detected. We suspected that sulfite is an intermediate, and we detected desulfoviridin (sulfite reductase). We thus believe that sulfonate reduction is one aspect of a respiratory system transferring electrons from, e.g., formate to sulfite reductase via an electron transport system which presumably generates a proton gradient across the cell membrane.

L7 ANSWER 13 OF 33 Elsevier BIOBASE on STN

ACCESSION NUMBER: 1997077607 ESBIOBASE <<LOGINID::20090224>>
TITLE: Stomatal responses to changes in air humidity are not necessarily linked to nocturnal CO.sub.2 uptake in the CAM plant Plectranthus marrubioides Benth. (Lamiaceae)
AUTHOR: Herppich W.B.
CORPORATE SOURCE: W.B. Herppich, WWU Munster, Intitut fur Okologie der Pflanzen, Hindenburgplatz 55, D-48143 Munster, Germany.
SOURCE: Plant, Cell and Environment, (1997), 20/3 (393-399), 26 reference(s)
CODEN: PLCEDV ISSN: 0140-7791
DOCUMENT TYPE: Journal; Article
COUNTRY: United Kingdom
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Plants of the crassulacean acid metabolism (CAM) species Plectranthus marrubioides (Lamiaceae) were subjected to short- and long-term changes in air humidity in controlled-environment experiments. Stomata of well-watered individuals of this all-cell leaf-succulent taxon responded directly, quickly and reversibly to variations of the water vapour gradient between leaf and air (Δw). Mean night-time leaf conductance to water vapour decreased curvilinearly with increasing Δw but linearly with lowered relative air humidity. Stomatal response was generally independent of the prevailing temperature and was not linked to CO.sub.2 uptake rates. Therefore, net night-time carbon gain, nocturnal malic acid accumulation and, thus, relative carbon recycling were not influenced by changes in air humidity in the temperature range tested. Mean nocturnal molar water use efficiency, however, decreased with decreasing air humidity because of the increased transpirational water loss. If watering was repeatedly withheld for several days during the experiments, employing a temperature regime of 35/30 °C day and night, stomatal conductance became low enough to inhibit CO.sub.2 uptake, but only at the

highest Δw. The results suggest that drought stress was necessary to increase responsiveness of plants to the point where CAM was also inhibited by decreases in air humidity.

L7 ANSWER 14 OF 33 Elsevier BIOBASE on STN

ACCESSION NUMBER: 1997061840 ESBIOBASE <<LOGINID::20090224>>
TITLE: GCAC1 recognizes the pH gradient across the plasma membrane: A pH-sensitive and ATP-dependent anion channel links guard cell membrane potential to acid and energy metabolism
AUTHOR: Schulz-Lessdorf B.; Lohse G.; Hedrich R.
CORPORATE SOURCE: R. Hedrich, LMPB, Julius-von-Sachs-Institut, Biozentrum Universitat Wurzburg, Mittlerer Dallenbergweg 64, 97082 Wurzburg, Germany.
SOURCE: Plant Journal, (1996), 10/6 (993-1004), 64 reference(s)
CODEN: PLJUED ISSN: 0960-7412
DOCUMENT TYPE: Journal; Article
COUNTRY: United Kingdom
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Ion channels in the plasma membrane of guard cells provide key mechanisms in signal perception and volume regulation during stomatal movement. Recent studies have suggested that the strongly voltage-dependent, inactivating guard cell anion channel (GCAC1) acts as a sensor of the ambient extracellular CO.sub.2 concentration and as a target of modulation by nucleotides and Ca.sup.2.sup.+ ions. Applying the patch-clamp technique it is demonstrated here that GCAC1 is activated by cytoplasmic ATP in a pH-dependent manner. When the apoplastic pH was buffered to 5.6 and the cytosolic pH dropped step-wise from 7.8 to 5.6, the single -channel activity increased as a function of proton concentration. This pH-sensitivity is characterized by a titratable site with an apparent pK value around 6.9. While the steepness and direction of the transmembrane pH gradient did not affect the kinetics of activation, deactivation and fast inactivation of the whole-cell anion current, the kinetics of slow inactivation and reactivation were strongly influenced. When at a constant intracellular proton concentration of pH 7.2 the external pH decreased from 7.2 to 5.6 the time constants of slow inactivation and the half-times of reactivation increased two- and sevenfold, respectively. The mechanism of nucleotide activation of GCAC1 was quantitatively analysed on the level of single-channel events. Using inside-out, cell-free membrane patches, GCAC1 half-activated around 0.4 mM ATP. The sigmoidal dose-dependence of anion channel activation could be well fitted with an apparent Hill coefficient of 3.6. This behaviour might indicate that the activation process involves a strongly cooperative interaction of four ATP-binding sites. Neither ATP nor its non-hydrolysable analogue AMP-PMP, which also activated GCAC1, altered the voltage-dependent gating. AMP-PMP stimulation and the insensitivity of GCAC1 towards the phosphatase inhibitor, okadaic acid, and the kinase inhibitors, staurosporine and H-7, provided evidence that nucleotide binding rather than phosphorylation caused channel activation. Since the time- and voltage-dependent activity of GCAC1 is strongly modulated by ATP and protons, this channel is capable of sensing changes in the energy status, acid metabolism and the H.sup.+ ATPase activity of guard cells.

L7 ANSWER 15 OF 33 Elsevier BIOBASE on STN

ACCESSION NUMBER: 1995014035 ESBIOBASE <<LOGINID::20090224>>

TITLE: Human DNA helicase II: A novel DNA unwinding enzyme identified as the Ku autoantigen

AUTHOR: Tuteja N.; Tuteja R.; Ochem A.; Taneja P.; Huang N.W.; Simoncsits A.; Susic S.; Rahman K.; Marusic L.; Chen J.; Zhang J.; Wang S.; Pongor S.; Falaschi A.

CORPORATE SOURCE: N. Tuteja, Int Ctr Genetic Engn Biotechnology, Area Science Park, Padriciano 99, I-34012 Trieste, Italy.

SOURCE: EMBO Journal, (1994), 13/20 (4991-5001)
CODEN: EMJODG ISSN: 0261-4189

DOCUMENT TYPE: Journal; Article
COUNTRY: United Kingdom
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Human DNA helicase II (HDH II) is a novel ATP-dependent DNA unwinding enzyme, purified to apparent homogeneity from HeLa cells, which (i) unwinds exclusively DNA duplexes, (ii) prefers partially unwound substrates and (iii) proceeds in the 3' to 5' direction on the bound strand. HDH II is a heterodimer of 72 and 87 kDa polypeptides. It shows single-stranded DNA-dependent ATPase activity, as well as double-stranded DNA binding capacity. All these activities comigrate in gel filtration and glycerol gradients, giving a sedimentation coefficient of 7.4S and a Stokes radius of .apprx.46 Å, corresponding to a native molecular weight of 158 kDa. The antibodies raised in rabbit against either polypeptide can remove from the solution all the activities of HDH II. Photoaffinity labelling with α-.sup.3.sup.2P ATP labelled both polypeptides. Microsequencing of the separate polypeptides of HDH II and cross-reaction with specific antibodies showed that this enzyme is identical to Ku, an autoantigen recognized by the sera of scleroderma and lupus erythematosus patients, which binds specifically to duplex DNA ends and is a regulator of a DNA-dependent protein kinase. Recombinant HDH II/Ku protein expressed in and purified from Escherichia coli cells showed DNA binding and helicase activities indistinguishable from those of the isolated protein. The exclusively nuclear location of HDH II/Ku antigen, its highly specific affinity for double-stranded DNA, its abundance and its newly demonstrated ability to unwind exclusively DNA duplexes, point to an additional, if still unclear, role for this molecule in DNA metabolism.

ACCESSION NUMBER: 1994085647 ESBIOBASE <<LOGINID::20090224>>

TITLE: The diverse Michaelis constants and maximum velocities of lactate dehydrogenase in situ in various types of cell

AUTHOR: Nakaei Y.; Stoward P.J.

CORPORATE SOURCE: Y. Nakaei, Department of Oral Anatomy, School of Dentistry, Tokushima University, 3 Kuramoto-cho, Tokushima 770, Japan.

SOURCE: Histochemical Journal, (1994), 26/4 (292-297)
CODEN: HISJAE ISSN: 0018-2214

DOCUMENT TYPE: Journal; Article
COUNTRY: United Kingdom
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The kinetics of lactate dehydrogenase in mouse cardiac muscle fibres, skeletal muscle fibres, gastric parietal cells, parotid gland ductal and acinar cells, oocytes and mouse and human hepatocytes were studied as a function of substrate concentration in

sections of unfixed mouse and human tissues incubated at 37°C on lactate agarose gel films. The absorbances of the final reaction products deposited in single cells of various types were measured continuously as a function of incubation time using an image analysis system. The initial velocities (v(i)) of the dehydrogenase were calculated from two equations deduced previously by us, v(i) = a.sub.1°A (equation 1) and v(i) = v + a.sub.2°A (equation 2), where v and °A are, respectively, the gradient (steady-state velocity) and intercept of the linear regression line of absorbance on time for incubation times between 1 and 3 min, and a.sub.1 and a.sub.2 are constants characteristic for each cell type. Hanes plots using v; calculated from equation 2 gave more consistent estimates of the Michaelis constant (Km) and the maximum reaction velocity (V(max)) than those employing either steady-state velocity measurements or v(i) calculated from equation 1. The K(m) thus found for mouse skeletal muscle fibres (10.4-12.5 mM) and hepatocytes (14.3-16.7 mM) agreed well with values determined previously in biochemical assays. However, the Km for cardiac muscle fibres (13.4 mM) was higher. The K(m)) of the enzyme in gastric parietal cells, parotid gland cells and oocytes was in the range 7.6-9.7 mM. The V(max) were more diverse, ranging from 29 μmoles hydrogen equivalents/cm.sup.3 cytoplasm/min units in mouse parotid gland acinar cells, 59-68 units in skeletal and cardiac muscle fibres, 62-65 units in gastric parietal cells and oocytes, and 102-110 units in hepatocytes. The diversity found for Km and V in different cell types confirms the value of the quantitative histochemical approach in revealing the heterogeneity of cellular metabolism in situ.

L7 ANSWER 17 OF 33 IFIPAT COPYRIGHT 2009 IFI on STN

AN: 10356153 IFIPAT;IFIUDB;IFICDB <<LOGINID::20090224>>

TITLE: Immunomodulation and effect on cell processes relating to serotonin family receptors; Inducting apoptosis; for treatment of autoimmune and neurodegenerative diseases

INVENTOR(S):
Albert; Ross, Philadelphia, PA, US
Davidson; Harold Carter, Philadelphia, PA, US
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Tretiakova; Anna A., Philadelphia, PA, US

PATENT ASSIGNEE(S): Unassigned

PATENT ASSIGNEE PROBABLE: Philadelphia Health and Education Corp (Probable)

AGENT: MORGAN, LEWIS & BOCKIUS LLP, 1701 MARKET STREET, PHILADELPHIA, PA, 19103-2921, US

	NUMBER	PK	DATE
PATENT INFORMATION:	US 20030100570	A1	20030529
APPLICATION INFORMATION:	US 2002-112261		20020329

	NUMBER	DATE	
PRIORITY APPLN. INFO.:	US 2001-280296P	20010330	(Provisional)
	US 2001-345295P	20011025	(Provisional)
	US 2002-353883P	20020131	(Provisional)
	US 2001-280296P	20010330	(Provisional)
	US 2001-345295P	20011025	(Provisional)
	US 2002-353883P	20020131	(Provisional)
FAMILY INFORMATION:	US 20030100570	20030529	
	US 6979447	20051227	

DOCUMENT TYPE: Utility
Patent Application - First Publication

FILE SEGMENT: CHEMICAL
APPLICATION
ENTRY DATE: Entered STN: 30 May 2003
Last Updated on STN: 19 May 2004

GOVERNMENT INTEREST:

(0002) This research was supported in part by U.S. Government funds (RO1 NS37726), and the U.S. Government may therefore have certain rights in the invention.

PARENT CASE DATA:

The present application is entitled to priority under 35 U.S.C. section 119(e), to U.S. Provisional Application No. 60/353,883, filed on Jan. 31, 2002, U.S. Provisional Application No. 60/345, 295, filed on Oct. 25, 2001, and U.S. Provisional Application No. 60/280,296, filed on Mar. 30, 2001, all of which are hereby incorporated by reference in their entirety herein.

NUMBER OF CLAIMS: 72 41 Figure(s).

DESCRIPTION OF FIGURES:

FIG. 1 is a diagram depicting the effects of macrophageconditioned media on the proliferation response of lymphocytes to a mitogenic activation signal.
FIG. 2 is a diagram depicting the major metabolic pathway of serotonin synthesis and degradation. The compound names are shown to the left of the structures, while the enzymes catalyzing the individual reactions are shown to the right.
FIG. 3 is a diagram depicting the effects of a tryptophan hydrolase inhibitor (para-chlorophenylalanine, PCPA) on mitogenic stimulation of human lymphocytes. That is, human peripheral blood lymphocytes (PBLs) were stimulated by the addition of 1 mu g/ml ConA.
FIG. 4 is a diagram depicting the effects of serotonin, tryptophan, or phenelzine on the activation of human PBLs stimulated with ConA. The assay was harvested at the time points indicating on the graph. The reagents were added at a concentration of 400 mu M.
FIG. 5, comprising three panels, is a diagram depicting the effects of tryptophan (trp), serotonin (5-HT), and phenelzine (Pz) on the mitogenic stimulation of human T cells at differing concentrations of ConA.
That is, FIG. 5A depicts the effects at 0.1 mu g/ml of ConA; FIG. 5B depicts the effects at 1 mu g/ml of ConA, and FIG. 5C depicts the effects of 10 mu g/ml of ConA. The dotted line in each of the panels refers to the baseline stimulation level of ConA without any added reagents.
FIG. 6 is a diagram depicting the effects of tryptophan and serotonin addition to phenelzine induced inhibition of activated lymphocytes. The ***individual*** reagents (Pz, Trp, and 5HT) were added at a concentration of 100 mu M.
FIG. 7A is a diagram depicting the dose-response effects of titrating a panel of agonists and antagonists known to be selective for the 5-HTR 1 receptors on the activation of ConA (5 mu g/ml) stimulated human lymphocytes. The ***cells*** were harvested 72 hours after initiating ConA stimulation. The drugs used for this study have the following well-defined attributes:
(R) 8-OH DPAT: a selective agonist for the 5HT 1A receptor; WAY 100635: a selective antagonist for the 5HT 1A receptor; Propranolol: a general 5HT 1 receptor antagonist as well as a beta-adrenergic antagonist; L 694247: a selective 1B/1D agonist; GR 55562: a selective 1B/1D antagonist; SB 216641: a selective 1B antagonist; and BRL 15522: a selective 1D antagonist; BRL 54443: a selective 1E/1F agonist.
FIG. 7B is a diagram depicting the dose-response effects of titrating a panel of agonists and antagonists known to be selective for the 5-HTR 1 receptors on the allogeneic stimulation of human lymphocytes (otherwise known as a mixed lymphocyte reaction). The cells were harvested 120 hours after the initiating stimulation. The drugs used for this study have the following

well -defined attributes: (R) 8-OH DPAT: a selective agonist for the 5HT 1A receptor; WAY 100635: a selective antagonist for the 5HT 1A receptor; Propranolol: a general 5HT 1 receptor antagonist as well as a beta-adrenergic antagonist; L 694247: a selective 1B/1D agonist; GR 55562: a selective 1B/1D antagonist; SB 216641: a selective 1B antagonist; BRL 15522: a selective 1D antagonist; BRL 54443: a selective 1E/1F agonist.

FIG. 8A is a diagram depicting the dose-response effects of titrating a panel of agonists and antagonists known to target the 5-HTR 2 receptors on the activation of ConA (5 mu g/ml) stimulated human lymphocytes. The cells were harvested 72 hours after initiating ConA stimulation. The drugs used for this study have the following well-defined attributes: DOI: 5HT 2 agonist (prolonged exposure of the receptors to this compound results in their down-regulation); LY 53857: selective 5HT2A/2B/ 2C antagonist; MDL 11939: selective 5HT 2A antagonist; SB 206553: selective 5HT2B/2C antagonist; SB 242084: selective 5HT 2C antagonist; Methysergide: partial type 1 agonist/type 2 antagonist; Methiothepin: general type 1, 2, 6 & 7 antagonist.

FIG. 8B is a diagram depicting the dose-response effects of titrating a panel of agonists and antagonists known to target the 5-HTR 2 receptors on the allogeneic stimulation of human lymphocytes (otherwise known as a mixed lymphocyte reaction). The cells were harvested 120 hours after the initiating stimulation. The drugs used for this study have the following ***well*** -defined attributes: DOI: 5HT 2 agonist (prolonged exposure of the receptors to this compound results in their downregulation); LY 53857: selective 5HT2A/2B/2C antagonist; MDL 11939: selective 5HT 2A antagonist; SB 206553: selective 5HT2B/ 2C antagonist; SB 242084: selective 5HT 2C antagonist; Methysergide: partial type 1 agonist/type 2 antagonist; Methiothepin: general type 1, 2, 6 and 7 antagonist.

FIG. 9A is a diagram depicting the dose-response effects of titrating a panel of agonists and antagonists known to target either the 5-HTR 3, 4, 6 or 7 receptors on the activation of ConA (5 mu g/ml) stimulated human lymphocytes. The cells were harvested 72 hours after initiating ConA stimulation. The drugs used for this study have the following well-defined attributes: SR 57222A: selective 5HT 3 agonist; Troposetron: selective 5HT 3 antagonist (clinically approved as an anti-emetic); RS 67333: selective 5HT4 agonist (down-regulates the receptors upon prolonged contact); SB 204070: selective 5HT 4 receptor antagonist; Ro 047690: selective 5HT 6 antagonist SB 269970: selective 5HT 7 antagonist.

FIG. 9B is a diagram depicting the dose-response effects of titrating a panel of agonists and antagonists known to target either the 5-HTR 3, 4, 6 or 7 receptors on the allogeneic stimulation of human lymphocytes (otherwise known as a mixed lymphocyte reaction). The cells were harvested 120 hours after the initiating stimulation. The drugs used for this study have the following well-defined attributes: SR 57222A: selective 5HT 3 agonist; Troposetron: selective 5HT 3 antagonist (clinically approved as an anti-emetic); RS 67333: selective 5HT4 agonist (down-regulates the receptors upon prolonged contact); SB 204070: selective 5HT 4 receptor antagonist; Ro 047690: selective 5HT 6 antagonist; SB 269970: selective 5HT 7 antagonist.

FIG. 10 is a graph depicting a murine mixed lymphocyte reaction assay (BALB/c vs. C57BL6) examining the effects of a 5HT3R agonist and a selective 5HT6R antagonist relative to the action of the 5HT1R agonist.

FIG. 11 is a diagram depicting the effect of a 5HT type 1 receptor antagonist and a 5HT type 2 receptor antagonist on the cell numbers occurring during the mitogenic stimulation of human lymphocyte activation. The ***cells*** were stimulated with 10 mu g/ml ConA. The cells were repurified on a Ficoll gradient prior to addition of the inhibitor. Trypan blue exclusion was used to count the viable cells.

FIG. 12A is a diagram depicting the effects of a highly selective 5HT type 2 receptor antagonist, LY 53857, on the mitogenic stimulation of human lymphocytes (ConA stimulation at 1 mu g/ml, and the cells were harvested at 72 hours). The results depict the effect of adding the inhibitor at time=0 or at 48 hours after the initiation of the assay.

FIG. 12B is a diagram depicting the effects of a highly selective 5HT type 2 receptor antagonist, SB 206553, on the mitogenic stimulation of human lymphocytes (ConA stimulation at 1 mu g/ml, and the cells were harvested at 72 hours). The diagram depicts the effect of adding the inhibitor at time=0 or at 48 hours after the initiation of the assay.
FIG. 12C is a diagram depicting the effects of a highly selective 5HT type 2 receptor antagonist, MDL 11939, on the mitogenic stimulation of human lymphocytes (ConA stimulation at 1 mu g/ml, and the cells were harvested at 72 hours). The data depicted the effect of adding the inhibitor at time=0 or at 48 hours after the initiation of the assay.
FIG. 12D is a diagram depicting the effects of a highly selective 5HT type 2 receptor antagonist, SB 242084, on the mitogenic stimulation of human lymphocytes (ConA stimulation at 1 mu g/ml, and the cells were harvested at 72 hours). The diagram depicts the effect of adding the inhibitor at time=0 or at 48 hours after the initiation of the assay.
FIG. 13 is a graph depicting the results of a murine allograft model for studying the effects of serotonin receptor antagonists versus Cyclosporin A. The date disclosed herein are derived from a cytotoxic T cell killing assay using the splenocytes from the treated mice versus the p815 target ***cells.***
FIG. 14 is a diagram depicting the effects of the 5HT2R selective antagonist, SB 206553, in a murine allograft model. The three SB 206553-treated mice were designated SB#226h, SB#226i, and SB#226j. Two of the treated mice had the allogeneic response completely suppressed. Only one of the mice (SB#226j) demonstrated virtually no immunologic effect as a result of treatment. However, SB#226j required repeated tail vein injections in order to administer the drug. Even when injected with great care, the tail vein injection can be technically difficult, and does not always occur on the first attempt.
FIG. 15 is an image depicting a gel demonstrating RT PCR priming of resting and activated lymphocytes and monocytes. The (+) lanes indicate cells that were mitogenically stimulated for 48 hours with ConA prior to creating a cDNA library. The (-) lanes indicate resting cell.

DESCRIPTION OF FIGURES:

FIG. 16 is an image depicting a Southern blot demonstrating expression of each of the fourteen distinct serotonin receptors, wherein the blots were probed with an appropriate internal oligonucleotide as follows: 1A: ctgcagaacgtggccaattatcttattggctcttt (SEQ ID NO: 1); 1B: gtggagtactcagctaaaaggactcccaagaggg (SEQ ID NO:2); 1D: ctctcttttcaaccacgtgaaaatcaagcttgct (SEQ ID NO: 3); 1E: atctagatcacccaggagaacgtcagcagatctcta (SEQ ID NO: 4); 1F: gagcagcaaagacattataccacaagagacaagcaa (SEQ ID NO: 5); 2A: tcggctcttttgtgtcatttttcattcccttaacca (SEQ ID NO: 6); 2B: ctcaacgcctaacatggttgactgtgtctacagttt (SEQ ID NO: 7); 2C: taactgacattttcaatacctccgatggtggacgct (SEQ ID NO: 8); 3A: gggagttcagcatggaaagcagtaactactatgcag (SEQ ID NO: 9); 3B: ttcaatctatcagcaactacctccaaactcaggacc (SEQ ID NO: 10); 4: caccattctttgtcaccaatattgtggatcctttc (SEQ ID NO: 11); 5: ctttttggctggggagagacgtactctgagg (SEQ ID NO: 12); 6: atcctcaacctctgcctcatcagcctggac (SEQ ID NO: 13); 7: tgaaaggaaaaacatctccatctttaagcgagaaca (SEQ ID NO: 14).
FIG. 17 is a graph depicting the functional behavior of various 5-HT Class 1 selective drugs. 8-OH DPAT is a 1A selective agonist; WAY 100635 is a selective 1A antagonist; propranolol is a general type 1 receptor antagonist (as well as a betaadrenergic antagonist); SB 216641 is a selective 1B antagonist; L694247 is a selective 1B/1D agonist; GR 55562 is a selective 1B/1D antagonist; BRL 54443 is a selective 1E/1F agonist. The drugs were added at time=0 of a 5mg/ml ConA stimulation of human lymphocytes as described elsewhere herein.
FIG. 18A is a graph depicting the data obtained using a murine allograft model described elsewhere herein. Briefly, the data depicted were obtained using a single representative study. The two positive controls shown indicate the observed the induced cytotoxic killing activity, whereas the naive controls

have never received the P815 cells and, consequently, provide a measure of the background of the assay. The Methysergidetreated (MS) mice demonstrate complete inhibition of the induced killing response.
FIG. 18B is a graph depicting data obtained using a nilurine allograft model as described elsewhere herein. The data depicted represents the pooled result of multiple assays, where the 100:1 effector:target ratio data was used to calculate the per cent inhibition. Each individual bar represents the data collected from a single mouse.
FIG. 19A is a graph depicting the effects on the 5-HT2A/B/C receptor antagonist LY53587 on RPMI 8226 cell viability at 16 hours.
FIG. 19B is a graph depicting the effects on fthe 5-HT2A/B/C receptor antagonist LY53587 on RPMI 8226 cell viability at 48 hours.
FIG. 20A is a graph depicting the effects of the 5HT-2A/B/C receptor antagonist of mitochondrial activity in RPMI 8226 cells at 16 hours.
FIG. 20B is a graph depicting the effects of the 5HT-2A/B/C receptor antagonist of mitochondrial activity in RPMI 8226 cells at 48 hours.
FIG. 20C is a graph depicting the effects of the 5HT-2A/B/C receptor antagonist of DNA synthesis in RPMI 8226 cells at 16 hours.
FIG. 20D is a graph depicting the effects of the 5HT-2A/B/C receptor antagonist of DNA synthesis in RPMI 8226 cells at 48 hours.
FIG. 21 is a graph depicting the effects of the 5HT-2A/B/C receptor antagonist of DNA synthesis in RPMI 8226 cells.
FIG. 22 is a graph depicting the effects of various 5-HTreceptor agonists and antagonists on RPMI 8226 cell proliferation.
FIG. 23 is a graph depicting the effects of various 5-HT receptor agonists and antagonist targeted to the 5-HTR 1 receptors. The readout of the assay is the cell proliferation of the RPMI 8226 multiple myeloma cells.
FIG. 24 is a graph depicting the effects of various 5-HT receptor agonists and antagonists targeted to the 5-HTR 2 receptors. The readout of the assay is the cell proliferation of the RPMI 8226 multiple myeloma cells.
FIG. 25 is a graph depicting the effects of various 5-HT receptor agonists and antagonists targeted to either the 5-HTR 3, 4, 6 or 7 receptors. The readout of the assay is the cell proliferation of the RPMI 8226 multiple myeloma cells.
FIG. 26 is an image depicting a gel demonstrating classical DNA fragmentation associated with apoptosis in RPMI 8226 cells treated with various agents, including a 5-HTR 2A/2B/2C.
FIG. 27, comprising panels A-F, is an image depicting the FACS profiles of RPMI 8226 cells treated with various concentrations of camptothecin, a selective 5-HTR type 1B/D antagonist, or untreated control cells all stained with annexin (along the ordinate) and propedium iodide (PI) (along the abscissa).
FIG. 28 are 4 images depicting matched Hematoxylin and eosin (top) and bis-benzamide (bottom) stained images of RPMI-8226 cells after 9 hour treatments with 2 mu M camptothecin (left) and 50 mu M SB 216641-treated to inhibit the 5-HT 1B receptor signals (right). Extensive chromatin condensation and nuclear fragmentation is evident in both treatment groups, indicative of widespread apoptosis.
FIG. 29 depicts a matched images of RPMI-8226 cells stained with Hematoxylin and eosin (top) and bis-benzamide (bottom) after 9 hour treatments with 50 mu M SB242084-treated to inhibit the 5HT 2C receptors signals (right) and vehicle control (left). Homogeneous chromatin-staining is apparent in the control sample, indicative of viable cells, whereas cells treated with SB242084 demonstrated condensed and fragmented chromatin, indicative of apoptotic cells.
FIG. 30, comprising panels A-D, is an image depicting a photomicrograph demonstrating the detectable changes in a cell upon inhibition of serotonergic signalling. The cells were incubated in the presence of a selective type 1B antagonist (SB 216641) and the changes in cell morphology are depicted after 24 hours of treatment.!

AB The present invention relates to the discovery that signaling via a serotonin type 1B, 2, 4 and 6 receptor is important in T cell activation such that inhibiting such signaling can be used to modulate the immune response. This immunomodulation is useful for the treatment of immune

diseases or conditions, and for the development of potential therapeutics for such diseases or conditions. It has been further discovered that, in cells proceeding through the cell cycle process, inhibition of serotonin signaling inhibits the process and induces apoptosis and morphological changes to a cell. These effects of inhibiting serotonergic signaling can be useful for effecting selective cell killing and for identifying compounds that inhibit the signaling.

CLMN 72 41 Figure(s).

FIG. 1 is a diagram depicting the effects of macrophageconditioned media on the proliferation response of lymphocytes to a mitogenic activation signal.

FIG. 2 is a diagram depicting the major metabolic pathway of serotonin synthesis and degradation. The compound names are shown to the left of the structures, while the enzymes catalyzing the individual reactions are shown to the right.

FIG. 3 is a diagram depicting the effects of a tryptophan hydrolase inhibitor (para-chlorophenylalanine, PCPA) on mitogenic stimulation of human lymphocytes. That is, human peripheral blood lymphocytes (PBLs) were stimulated by the addition of 1 mu g/ml ConA.

FIG. 4 is a diagram depicting the effects of serotonin, tryptophan, or phenelzine on the activation of human PBLs stimulated with ConA. The assay was harvested at the time points indicating on the graph. The reagents were added at a concentration of 400 mu M.

FIG. 5, comprising three panels, is a diagram depicting the effects of tryptophan (trp), serotonin (5-HT), and phenelzine (Pz) on the mitogenic stimulation of human T cells at differing concentrations of ConA. That is, FIG. 5A depicts the effects at 0.1 mu g/ml of ConA; FIG. 5B depicts the effects at 1 mu g/ml of ConA, and FIG. 5C depicts the effects of 10 mu g/ml of ConA. The dotted line in each of the panels refers to the baseline stimulation level of ConA without any added reagents.

FIG. 6 is a diagram depicting the effects of tryptophan and serotonin addition to phenelzine induced inhibition of activated lymphocytes. The individual reagents (Pz, Trp, and 5HT) were added at a concentration of 100 mu M.

FIG. 7A is a diagram depicting the dose-response effects of titrating a panel of agonists and antagonists known to be selective for the 5-HTR 1 receptors on the activation of ConA (5 mu g/ml) stimulated human lymphocytes. The cells were harvested 72 hours after initiating ConA stimulation. The drugs used for this study have the following well-defined attributes: (R) 8-OH DPAT: a selective agonist for the 5HT 1A receptor; WAY 100635: a selective antagonist for the 5HT 1A receptor; Propranolol: a general 5HT 1 receptor antagonist as well as a beta-adrenergic antagonist; L 694247: a selective 1B/1D agonist; GR 55562: a selective 1B/1D antagonist; SB 216641: a selective 1B antagonist; and BRL 15522: a selective 1D antagonist; BRL 54443: a selective 1E/1F agonist.

FIG. 7B is a diagram depicting the dose-response effects of titrating a panel of agonists and antagonists known to be selective for the 5-HTR 1 receptors on the allogeneic stimulation of human lymphocytes (otherwise known as a mixed lymphocyte reaction). The cells were harvested 120 hours after the initiating stimulation. The drugs used for this study have the following well-defined attributes: (R) 8-OH DPAT: a selective agonist for the 5HT 1A receptor; WAY 100635: a selective antagonist for the 5HT 1A receptor; Propranolol: a general 5HT 1 receptor antagonist as well as a beta-adrenergic antagonist; L 694247: a selective 1B/1D agonist; GR 55562: a selective 1B/1D antagonist; SB 216641: a selective 1B antagonist; BRL 15522: a selective 1D antagonist; BRL 54443: a selective 1E/1F agonist.

FIG. 8A is a diagram depicting the dose-response effects of titrating a panel of agonists and antagonists known to target the 5-HTR 2 receptors

on the activation of ConA (5 mu g/ml) stimulated human lymphocytes. The cells were harvested 72 hours after initiating ConA stimulation. The drugs used for this study have the following well-defined attributes: DOI: 5HT 2 agonist (prolonged exposure of the receptors to this compound results in their down-regulation); LY 53857: selective 5HT2A/2B/ 2C antagonist; MDL 11939: selective 5HT 2A antagonist; SB 206553: selective 5HT2B/2C antagonist; SB 242084: selective 5HT 2C antagonist; Methysergide: partial type 1 agonist/type 2 antagonist; Methiothepin: general type 1, 2, 6 & 7 antagonist.

FIG. 8B is a diagram depicting the dose-response effects of titrating a panel of agonists and antagonists known to target the 5-HTR 2 receptors on the allogeneic stimulation of human lymphocytes (otherwise known as a mixed lymphocyte reaction). The cells were harvested 120 hours after the initiating stimulation. The drugs used for this study have the following well-defined attributes: DOI: 5HT 2 agonist (prolonged exposure of the receptors to this compound results in their downregulation); LY 53857: selective 5HT2A/2B/2C antagonist; MDL 11939: selective 5HT 2A antagonist; SB 206553: selective 5HT2B/ 2C antagonist; SB 242084: selective 5HT 2C antagonist; Methysergide: partial type 1 agonist/type 2 antagonist; Methiothepin: general type 1, 2, 6 and 7 antagonist.

FIG. 9A is a diagram depicting the dose-response effects of titrating a panel of agonists and antagonists known to target either the 5-HTR 3, 4, 6 or 7 receptors on the activation of ConA (5 mu g/ml) stimulated human lymphocytes. The cells were harvested 72 hours after initiating ConA stimulation. The drugs used for this study have the following well-defined attributes: SR 57222A: selective 5HT 3 agonist; Troposetron: selective 5HT 3 antagonist (clinically approved as an anti-emetic); RS 67333: selective 5HT4 agonist (down-regulates the receptors upon prolonged contact); SB 204070: selective 5HT 4 receptor antagonist; Ro 047690: selective 5HT 6 antagonist SB 269970: selective 5HT 7 antagonist.

FIG. 9B is a diagram depicting the dose-response effects of titrating a panel of agonists and antagonists known to target either the 5-HTR 3, 4, 6 or 7 receptors on the allogeneic stimulation of human lymphocytes (otherwise known as a mixed lymphocyte reaction). The cells were harvested 120 hours after the initiating stimulation. The drugs used for this study have the following well-defined attributes: SR 57222A: selective 5HT 3 agonist; Troposetron: selective 5HT 3 antagonist (clinically approved as an anti-emetic); RS 67333: selective 5HT4 agonist (down-regulates the receptors upon prolonged contact); SB 204070: selective 5HT 4 receptor antagonist; Ro 047690: selective 5HT 6 antagonist; SB 269970: selective 5HT 7 antagonist.

FIG. 10 is a graph depicting a murine mixed lymphocyte reaction assay (BALB/c vs. C57BL6) examining the effects of a 5HT3R agonist and a selective 5HT6R antagonist relative to the action of the 5HT1R agonist.

FIG. 11 is a diagram depicting the effect of a 5HT type 1 receptor antagonist and a 5HT type 2 receptor antagonist on the cell numbers occurring during the mitogenic stimulation of human lymphocyte activation. The cells were stimulated with 10 mu g/ml ConA. The cells were repurified on a Ficoll gradient prior to addition of the inhibitor. Trypan blue exclusion was used to count the viable cells.

FIG. 12A is a diagram depicting the effects of a highly selective 5HT type 2 receptor antagonist, LY 53857, on the mitogenic stimulation of human lymphocytes (ConA stimulation at 1 mu g/ml, and the cells were harvested at 72 hours). The results depict the effect of adding the inhibitor at time=0 or at 48 hours after the initiation of the assay.

FIG. 12B is a diagram depicting the effects of a highly selective 5HT type 2 receptor antagonist, SB 206553, on the mitogenic stimulation of human lymphocytes (ConA stimulation at 1 mu g/ml, and the cells were

harvested at 72 hours). The diagram depicts the effect of adding the inhibitor at time=0 or at 48 hours after the initiation of the assay.

FIG. 12C is a diagram depicting the effects of a highly selective 5HT type 2 receptor antagonist, MDL 11939, on the mitogenic stimulation of human lymphocytes (ConA stimulation at 1 mu g/ml, and the cells were harvested at 72 hours). The data depicted the effect of adding the inhibitor at time=0 or at 48 hours after the initiation of the assay.

FIG. 12D is a diagram depicting the effects of a highly selective 5HT type 2 receptor antagonist, SB 242084, on the mitogenic stimulation of human lymphocytes (ConA stimulation at 1 mu g/ml, and the cells were harvested at 72 hours). The diagram depicts the effect of adding the inhibitor at time=0 or at 48 hours after the initiation of the assay.

FIG. 13 is a graph depicting the results of a murine allograft model for studying the effects of serotonin receptor antagonists versus Cyclosporin A. The date disclosed herein are derived from a cytotoxic T cell killing assay using the splenocytes from the treated mice versus the p815 target cells.

FIG. 14 is a diagram depicting the effects of the 5HT2R selective antagonist, SB 206553, in a murine allograft model. The three SB 206553-treated mice were designated SB#226h, SB#226i, and SB#226j. Two of the treated mice had the allogeneic response completely suppressed. Only one of the mice (SB#226j) demonstrated virtually no immunologic effect as a result of treatment. However, SB#226j required repeated tail vein injections in order to administer the drug. Even when injected with great care, the tail vein injection can be technically difficult, and does not always occur on the first attempt.

FIG. 15 is an image depicting a gel demonstrating RT PCR priming of resting and activated lymphocytes and monocytes. The (+) lanes indicate cells that were mitogenically stimulated for 48 hours with ConA prior to creating a cDNA library. The (-) lanes indicate resting cell.

FIG. 16 is an image depicting a Southern blot demonstrating expression of each of the fourteen distinct serotonin receptors, wherein the blots were probed with an appropriate internal oligonucleotide as follows: 1A: ctgcagaacgtggccaattatcttattggctcttt (SEQ ID NO: 1); 1B: gtggagtactcagctaaaaggactcccaagaggg (SEQ ID NO:2); 1D: ctctcttttcaaccacgtgaaaatcaagcttgct (SEQ ID NO: 3); 1E: atctagatcacccaggagaacgtcagcagatctcta (SEQ ID NO: 4); 1F: gagcagcaaagacattataccacaagagacaagcaa (SEQ ID NO: 5); 2A: tcggctcttttgtgtcatttttcattcccttaacca (SEQ ID NO: 6); 2B: ctcaacgcctaacatggttgactgtgtctacagttt (SEQ ID NO: 7); 2C: taactgacattttcaatacctccgatggtggacgct (SEQ ID NO: 8); 3A: gggagttcagcatggaaagcagtaactactatgcag (SEQ ID NO: 9); 3B: ttcaatctatcagcaactacctccaaactcaggacc (SEQ ID NO: 10); 4: caccattctttgtcaccaatattgtggatcctttc (SEQ ID NO: 11); 5: ctttttggctggggagagacgtactctgagg (SEQ ID NO: 12); 6: atcctcaacctctgcctcatcagcctggac (SEQ ID NO: 13); 7: tgaaaggaaaaacatctccatctttaagcgagaaca (SEQ ID NO: 14).

FIG. 17 is a graph depicting the functional behavior of various 5-HT Class 1 selective drugs. 8-OH DPAT is a 1A selective agonist; WAY 100635 is a selective 1A antagonist; propranolol is a general type 1 receptor antagonist (as well as a betaadrenergic antagonist); SB 216641 is a selective 1B antagonist; L694247 is a selective 1B/1D agonist; GR 55562 is a selective 1B/1D antagonist; BRL 54443 is a selective 1E/1F agonist. The drugs were added at time=0 of a 5mg/ml ConA stimulation of human lymphocytes as described elsewhere herein.

FIG. 18A is a graph depicting the data obtained using a murine allograft model described elsewhere herein. Briefly, the data depicted were obtained using a single representative study. The two positive controls shown indicate the observed the induced cytotoxic killing activity, whereas the naive controls have never received the P815 cells and,

consequently, provide a measure of the background of the assay. The Methysergidetreated (MS) mice demonstrate complete inhibition of the induced killing response.

FIG. 18B is a graph depicting data obtained using a nilurine allograft model as described elsewhere herein. The data depicted represents the pooled result of multiple assays, where the 100:1 effector:target ratio data was used to calculate the per cent inhibition. Each individual bar represents the data collected from a single mouse.

FIG. 19A is a graph depicting the effects on the 5-HT2A/B/C receptor antagonist LY53587 on RPMI 8226 cell viability at 16 hours.

FIG. 19B is a graph depicting the effects on fthe 5-HT2A/B/C receptor antagonist LY53587 on RPMI 8226 cell viability at 48 hours.

FIG. 20A is a graph depicting the effects of the 5HT-2A/B/C receptor antagonist of mitochondrial activity in RPMI 8226 cells at 16 hours.

FIG. 20B is a graph depicting the effects of the 5HT-2A/B/C receptor antagonist of mitochondrial activity in RPMI 8226 cells at 48 hours.

FIG. 20C is a graph depicting the effects of the 5HT-2A/B/C receptor antagonist of DNA synthesis in RPMI 8226 cells at 16 hours.

FIG. 20D is a graph depicting the effects of the 5HT-2A/B/C receptor antagonist of DNA synthesis in RPMI 8226 cells at 48 hours.

FIG. 21 is a graph depicting the effects of the 5HT-2A/B/C receptor antagonist of DNA synthesis in RPMI 8226 cells.

FIG. 22 is a graph depicting the effects of various 5-HTreceptor agonists and antagonists on RPMI 8226 cell proliferation.

FIG. 23 is a graph depicting the effects of various 5-HT receptor agonists and antagonist targeted to the 5-HTR 1 receptors. The readout of the assay is the cell proliferation of the RPMI 8226 multiple myeloma cells.

FIG. 24 is a graph depicting the effects of various 5-HT receptor agonists and antagonists targeted to the 5-HTR 2 receptors. The readout of the assay is the cell proliferation of the RPMI 8226 multiple myeloma cells.

FIG. 25 is a graph depicting the effects of various 5-HT receptor agonists and antagonists targeted to either the 5-HTR 3, 4, 6 or 7 receptors. The readout of the assay is the cell proliferation of the RPMI 8226 multiple myeloma cells.

FIG. 26 is an image depicting a gel demonstrating classical DNA fragmentation associated with apoptosis in RPMI 8226 cells treated with various agents, including a 5-HTR 2A/2B/2C.

FIG. 27, comprising panels A-F, is an image depicting the FACS profiles of RPMI 8226 cells treated with various concentrations of camptothecin, a selective 5-HTR type 1B/D antagonist, or untreated control cells all stained with annexin (along the ordinate) and propedium iodide (PI) (along the abscissa).

FIG. 28 are 4 images depicting matched Hematoxylin and eosin (top) and bis-benzamide (bottom) stained images of RPMI-8226 cells after 9 hour treatments with 2 mu M camptothecin (left) and 50 mu M SB 216641-treated to inhibit the 5-HT 1B receptor signals (right). Extensive chromatin condensation and nuclear fragmentation is evident in both treatment groups, indicative of widespread apoptosis.

FIG. 29 depicts a matched images of RPMI-8226 cells stained with Hematoxylin and eosin (top) and bis-benzamide (bottom) after 9 hour treatments with 50 mu M SB242084-treated to inhibit the 5HT 2C receptors signals (right) and vehicle control (left). Homogeneous chromatin-staining is apparent in the control sample, indicative of viable cells, whereas cells treated with SB242084 demonstrated condensed and fragmented chromatin, indicative of apoptotic cells.

FIG. 30, comprising panels A-D, is an image depicting a photomicrograph demonstrating the detectable changes in a cell upon inhibition of serotonergic signalling. The cells were incubated in the presence of a selective type 1B antagonist (SB 216641) and the changes in cell morphology are depicted after 24 hours of treatment.!

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TITLE: Polypeptides, their production and use; Polypeptide for use in the treatment of hypercholesterolemia, hyperlipemia, diabetes, cancer, pancreatitis, bone, nervvous system, kidney and sexual disorders

INVENTOR(S): Fukusumi; Shoji, Tsukuba-shi, JP
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PATENT ASSIGNEE(S): Unassigned

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	NUMBER	PK	DATE
PATENT INFORMATION:	US 20020143152	A1	20021003
APPLICATION INFORMATION:	US 2002-44592		20020110

	APPLN. NUMBER	DATE	GRANTED PATENT NO. OR STATUS
Section 371 PCT Filing OF:	WO 1998-JP1923	19980427	
CONTINUATION-IN-PART OF:	US 1999-403639	19991025	PENDING

	NUMBER	DATE
PRIORITY APPLN. INFO.:	JP 1997-109974	19970428
FAMILY INFORMATION:	US 20020143152	20021003

DOCUMENT TYPE: Utility
Patent Application - First Publication

FILE SEGMENT: CHEMICAL
APPLICATION

OTHER SOURCE: CA 137:274806

ENTRY DATE: Entered STN: 7 Oct 2002
Last Updated on STN: 27 Aug 2003

PARENT CASE DATA:

REFERENCE TO RELATED APPLICATIONS This application is a continuation-in-part of U.S. patent application Ser. No. 09/403,639, filed Oct. 25, 1999, which is the National Phase filing of International Application for Patent Ser. No. PCT/JP98/01923 filed Apr.27, 1998.

NUMBER OF CLAIMS: 12 41 Figure(s).

DESCRIPTION OF FIGURES:

FIG. 1 shows the nucleotide sequence of the human pituitaryderived G protein-coupled receptor protein cDNA fragment harbored in cDNA clone p19P2 isolated by PCR using human pituitary-derived cDNA (SEQ ID NO.:63) and the amino acid (SEQ ID NO.: 64) encoded by the nucleotide sequence. The primer used for sequencing was-21M13. The underscored region corresponds to the synthetic primer.

FIG. 2 shows the nucleotide sequence of the human pituitaryderived G protein-coupled receptor protein cDNA fragment harbored in cDNA clone p19P2 isolated by PCR using human pituitary-derived cDNA (SEQ ID NO.: 65) and the amino acid sequence encoded thereby (SEQ ID NO.: 66). The primer used for sequencing was M13RV-N (Takara). The underscored region corresponds to the synthetic primer.

FIG. 3 shows a partial hydrophobic plot of the protein encoded by the human pituitary-derived G protein-coupled receptor protein cDNA fragment harbored in p19P2 constructed according to the amino acid sequence shown in FIG. 1.

FIG. 4 shows a partial hydrophobic plot of the protein encoded by the human pituitary-derived G protein-coupled receptor protein cDNA fragment harbored in p19P2 constructed according to the amino acid sequence shown in FIG. 2.

FIG. 5 is a diagram comparing the partial amino acid sequence of the protein encoded by the human pituitary-derived G proteincoupled receptor protein cDNA fragment harbored in p19P2 as shown in FIGS. 1 (SEQ ID NO.: 67) and FIG. 2 (SEQ ID NO.: 68), with the known G protein-coupled receptor protein S12863 (SEQ ID NO.: 69). The shadowed region represents the region of agreement. The 1st to 9th amino acid sequence of p19P2 corresponds to the 1st to 99th amino acid sequence of FIG. 1 and the 156th to 230th amino acid sequence corresponds to the 1st to 68th amino acid sequence of FIG. 2.

FIG. 6 shows the nucleotide sequence of the MIN6-derived G protein-coupled receptor protein cDNA fragment based on the nucleotide sequences of the MIN6-derived G protein-coupled receptor protein cDNA fragments harbored in the cDNA clones pG32 and pG1-10 isolated by PCR using MIN6-derived cDNA and the amino acid sequence (SEQ ID NO.: 71) encoded by the nucleotide sequence (SEQ ID NO.: 70). The underscored region corresponds to the synthetic primer.

FIG. 7 is a diagram comparing the partial amino acid sequence encoded by pG3-2/pG1-10 of the MIN6-derived G protein-coupled receptor protein shown in FIG. 6 (SEQ ID NO.: 72) with the partial amino acid sequence of the protein encoded by p19P2 shown in FIGS. 1 and 2. The shadowed region corresponds to the region of agreement. The 1st to 99th amino acid sequence of the protein encoded by p19P2 corresponds to the 1st to 99th amino acid sequence of FIG. 1 and the 156th to 223rd amino acid sequence corresponds to the 1st to 68th amino acid sequence of FIG. 2. The 1st to 223rd amino acid sequence of the protein encoded by pG3-2/pG1-10 corresponds to the 1st to 223rd amino acid sequence of FIG. 6.

FIG. 8 is a partial hydrophobic plot of the MIN6-derived G protein-coupled receptor protein constructed according to the partial amino acid sequence shown in FIG. 6.

FIG. 9 shows the entire nucleotide sequence of the human pituitary-derived G protein-coupled receptor protein cDNA harbored in the cDNA clone phGR3 isolated from a human pituitary-derived cDNA library by the plaque hybridization method using the DNA fragment inserted in p19P2 as a probe and the amino acid sequence (SEQ ID NO.: 74) encoded by the nucleotide sequence (SEQ ID NO.: 73).

FIG. 10 shows the results of Northern blotting of human pituitary mRNA hybridized with radioisotope-labeled human pituitary cDNA clone phGR3.

FIG. 11 shows a hydrophobic plot of the protein encoded by the human pituitary-derived G protein-coupled receptor protein cDNA harbored in the phGR3 as constructed according to the amino acid sequence shown in FIG. 9.

FIG. 12 shows the nucleotide sequence of the MIN6-derived G protein-coupled receptor protein cDNA fragment (SEQ ID NO.: 75) harbored in the cDNA clone p5S38 isolated by PCR using MIN6derived cDNA and the amino acid sequence (SEQ ID NO.: 76) encoded by the nucleotide sequence. The underscored region corresponds to the synthetic primer.

FIG. 13 shows a diagram comparing the partial amino acid sequence (SEQ ID NO.: 76) of MIN6-derived G protein-coupled receptor protein encoded by p5S38 shown in FIG. 12 with the partial amino acid sequence of G protein-coupled receptor protein encoded by the cDNA fragment harbored in p19P2 as shown in FIGS. 1 and 2 and the partial amino acid sequence of G protein-coupled receptor protein encoded by the nucleotide sequence generated from the nucleotide sequences of cDNA fragments contained in pG3-2 and pG1-10 shown in FIG. 6. The shadowed region represents the sequence region of agreement. The 1st to 144th amino acid sequence of the protein encoded by p5S38 corresponds to the 1st to 144th amino acid sequence of FIG. 12, the 1st to 99th amino acid sequence of the protein encoded by p19P2 corresponds to the 1st to 99th amino acid sequence of FIG. 1 and the 156th to 223rd amino acid sequence corresponds to 1st to 68th amino acid sequence of FIG. 2. The 1st to 223rd amino acid sequence of the protein encoded by pG32/pG1-10 corresponds to the 1st to 223rd amino acid sequence of FIG. 6.

FIG. 14 shows a partial hydrophobic plot of the protein encoded by the MIN6-derived G protein-coupled receptor protein cDNA harbored in p5S38 as

constructed according to the partial amino acid sequence shown in FIG. 12.
FIG. 15 shows the results of the following analysis. Thus, RTPCR was carried out to confirm the expression of mRNA in CHO cells transfected by PAKKO-19P2. Lanes 1-7 represent the results obtained by performing PCRs using serial dilutions of pAKKO-19P2 for comparison, i.e. the 10 mu l/ml stock solution (lane 1), 1/2 dilution (lane 2), 1/4 dilution (lane 3), (fraction (1/64)) dilution (Lane 4), (fraction (1/256)) dilution (lane 5), (fraction (1/1024)) dilution (lane 6), and (fraction (1/4096)) dilution (lane 7) of the plasmid as templates, and analyzing the reaction mixtures by 1.2% agarose gel electrophoresis. Lanes 8 through 11 are the results obtained by performing PCRs using a (fraction (1/10)) dilution (lane 8), a (fraction (1/100)) dilution (lane 9), and a (fraction (1/1000)) dilution (lane 10) of the cDNA prepared from the CHO-19P2 cell line as templates and subjecting the respective reaction mixtures to electrophoresis. Lane 11 was obtained by performing PCR using a template obtained by carrying out cDNA synthesis without reverse transcriptase and subjecting the PCR reaction product to electrophoresis. Lanes 12 and 13 were obtained by performing PCR using cDNAs prepared from mock CHO cells with and without addition of reverse transcriptase, respectively, as templates and subjecting the respective reaction products to electrophoresis. M represents the DNA size marker. The lanes at both ends were obtained by electrophoresing 1 mu l of lambda /Sty I digest (Nippon Gene) and the second lane from right was obtained with 1 mu l of theta / chi 174/Hinc II digest (Nippon Gene). The arrowmark indicates the position of the band amplified by PCR of about 400 bp.
FIG. 16 shows the activity of the crude ligand peptide fraction extracted from rat whole brain to promote release of arachidonic acid metabolites from CHO-19P2 cells. The arachidonic acid metabolite releasing activity was expressed as % of the amount of (3H) arachidonic acid metabolites released in the presence of the crude ligand polypeptide fraction with the amount of (3H) arachidonic acid metabolites released in the presence of 0.05% BAS-HABB being taken as 100%. The activity to promote release of arachidonic acid metabolites from the CHO19P2 cell line was detected in a 30% CH3CN fraction.
FIG. 17 shows the activity of the crude ligand polypeptide fraction extracted from bovine hypothalamus to promote release of arachidonic acid metabolites from CHO-19P2 cells. The arachidonic acid metabolite release-promoting activity was expressed as % of the amount of (3H) arachidonic acid metabolites released in the presence of the crude ligand polypeptide fraction with the amount of (3H) arachidonic acid metabolites released in the presence of 0.05% BAS-HABB being taken as 100%. The activity to promote release of arachidonic acid metabolites from the CHO-19P2 cell line was detected in a 30% CH3CN fraction just as in the crude ligand polypeptide fraction from rat whole brain.
FIG. 18 shows the activity of the fraction purified with the reversed-phase column C18 218TP5415 to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells. The active fraction from RESOURCE S was fractionated on C18 218TP5415. Thus, chromatography was carried out at a flow rate of 1 ml/min. on a concentration gradient of 20%-30% CH3CN/0.1% TFA/H2O, the eluate was collected in 1 ml fractions, and each fraction was lyophilized. Then, the activity of each fraction to specifically promote release of arachidonic acid metabolites from the CHO-19P2 cell line was determined. As a result, the activity was fractionated into 3 fractions (designated, in the order of elution, as P-1, P-2, and P-3).
FIG. 19 shows the activity of the fraction purified with the diphenyl 219TP5415 reversed-phase column to specifically promote arachidonic acid metabolite release from CHO-19P2 cells. The P-3 active fraction from C18 218TP5415 was fractionated on diphenyl 219TP5415. The chromatography was carried out at a flow rate of 1 ml/min. on a concentration gradient of 22%-25% CH3CN/0.1% TFA/H2O, the eluate was collected in 1 ml fractions, and each fraction was lyophilized. Then, the activity to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells in each fraction was determined. As a result, the activity converged in a single peak.

DESCRIPTION OF FIGURES:

FIG. 20 shows the activity of the fraction purified by mu RPC C2/ C18 SC 2.1/10

reversed-phase column to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells. The peak active fraction from diphenyl 219TP5415 was fractionated on mu RPC C2/C18 SC 2.1/10. The chromatography was carried out at a flow rate of 100 mu l/min. on a concentration gradient of 22%-23.5% CH3CN/0.1% TFA/H2O, the eluate was collected in 100 mu l fractions, and each fraction was lyophilized. Then, the activity to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells in each fraction was determined. As a result, the activity was found as two peaks of apparently a single substance (peptide).

FIG. 21 shows the activity of the P-2 fraction purified by mu RPC C2/C18 SC 2.1/10 reversed-phase column to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells. The chromatography was carried out at a flow rate of 100 mu l/min. on a concentration gradient of 21.5%-23.0% CH3CN/0.1% TFA/dH2O, the eluate was collected in 100 mu l fractions, and each fraction was lyophilized. Then, the activity to specifically promote release of arachidonic acid metabolites from CHO-19P2 ***cells***   in each fraction was determined. As a result, the activity was found as a peak of apparently a single substance.

FIG. 22 shows the nucleotide sequence of bovine hypothalamus ligand polypeptide cDNA fragment (SEQ ID NO.: 77) as derived from the nucleotide sequence of the bovine hypothalamus-derived ligand polypeptide cDNA fragment which specifically promotes release of arachidonic acid metabolites from CHO-19P2 cells as harbored in a cDNA clone isolated by PCR using bovine hypothalamus-derived cDNA and the amino acid sequence (SEQ ID NO.: 78) encoded by said nucleotide sequence. The region indicated by the arrowmark corresponds to the synthetic primer.

FIG. 23 shows the nucleotide sequence of the bovine hypothalamusderived ligand polypeptide cDNA fragment (SEQ ID NO.: 79) generated according to the nucleotide sequence of the bovine hypothalamus-derived ligand polypeptide cDNA fragment which specifically promotes release of arachidonic acid metabolites from CHO-19P2 cells as harbored in a cDNA clone isolated by PCR using bovine hypothalamus-derived cDNA and the amino acid sequence (SEQ ID NO.: 80) encoded by said nucleotide sequence. The region indicated by the arrowmark corresponds to the synthetic primer.

FIG. 24 shows the amino acid sequences of the bovine hypothalamus-derived ligand polypeptides which specifically promote release of arachidonic acid metabolites from CHO-19P2 cells; FIG. 24a (SEQ ID NO.: 82), FIG. 24b (SEQ ID NO.: 84), and the cDNA sequence coding for the full coding region of the ligand polypeptides defined by SEQ ID NO.: 1 and SEQ ID NO.: 44; FIG. 24a (SEQ ID NO.: 81), FIG. 24b (SEQ ID NO.: 83).

FIG. 25 shows the concentration-dependent activity of synthetic ligand polypeptide (19P2-L31) to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells. The synthetic peptide was dissolved in degassed d H2O at a final concentration of 10-3M and diluted with 0.05% BSA-HBSS to concentrations of 10-12M-10-6M. The arachidonic acid ***metabolite***   releasing activity was expressed in the measured radioactivity of (3H) arachidonic acid metabolites released in the supernatant when the dilution was added to the cells. As a result, the activity of 19P2-31 to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells was found in a concentration-dependent manner.

FIG. 26 shows the concentration-dependent activity of synthetic ligand polypeptide (19P2-L31(O)) to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells. The synthetic ligand peptide was dissolved in degassed d H2O at a final concentration of 10-3M and diluted with 0.05% BSA-HBSS to concentrations of 10-12M-10-6M. The arachidonic acid ***metabolite***   releasing activity was expressed in the measured radioactivity of (3H) arachidonic acid metabolites released in the supernatant when the dilution was added to the cells. As a result, the activity of 19P2-L31(O) to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells was found in a dose-dependent manner.

FIG. 27 shows the activity of synthetic ligand polypeptide 19P2L20 to specifically promote release of arachidonic acid metabolites from CHO-19P2 ***cells.*** The synthetic peptide was dissolved in degassed distilled water at a final concentration of 10-3M and diluted with 0.05% BSA-HBSS to concentrations of 10-12M-10-6M. The arachidonic acid metabolite releasing activity was expressed in the measured radioactivity of (3H) arachidonic acid metabolites released in the supernatant when the dilution was added to the cells. As a result, the activity of 19P2-L20 to specifically promote release of arachidonic acid metabolites from CHO-19P2 ***cells*** was found in a dose-dependent manner.
FIG. 28 shows the 1.2% agarose gel electrophoregram of the DNA fragments of the phages cloned from a bovine genomic library as digested with restriction enzymes BamHI(B) and SalI(S). As the DNA size marker (M), StyI digests of lambda phage DNA were used. In lane B, two bands derived from the vector were detected in positions between the first (19,329 bp) and second (7.743 bp) marker bands, as well as 3 bands derived from the inserted fragment between the third (6,223 bp) and 5th (3,472 bp) bands. In lane S, two bands derived from the vector were similarly detected but due to the overlap of the band of the inserted fragment, the upper band is thicker than the band in lane B.
FIG. 29 shows the nucleotide sequence around the coding region as decoded from bovine genomic DNA (SEQ ID NO.: 85). The 1st to 3rd bases (ATG) correspond to the translation start codon and the 767th to 769th bases (TAA) correspond to the translation end codon.
FIG. 30 shows a comparison between the nucleotide sequence (genome) around the coding region as deduced from bovine genomic DNA (SEQ ID NO.: 85) and the nucleotide sequence (cDNA) of bovine cDNA cloned by PCR (SEQ ID NO. :87). The sequence region of agreement is indicated by shading. As to the 101st to 572nd region, there is no corresponding region in the nucleotide sequence of cDNA, indicating that it is an intron.
FIG. 31 shows the translation of the amino acid sequence (SEQ ID NO.: 88) encoded after elimination of the intron from the nucleotide sequence around the coding region as decoded from bovine genomic DNA (SEQ ID NO.: 87).
FIG. 32 shows the full-length amino acid sequence (SEQ ID NO.: 90) and the cDNA sequence coding for the full coding region of rat ligand polypeptide (SEQ ID NO.:89).
FIG. 33 shows the amino acid sequence of bovine ligand polypeptide (SEQ ID NO.: 88) and the nucleotide sequences of DNAs coding for bovine polypeptide (SEQ ID NO.: 87) and rat polypeptide (SEQ ID NO.: 89). The arrowmark indicates the region corresponding to the synthetic primer.
FIG. 34 shows the full-length amino acid sequence and the sequence of cDNA (SEQ ID NO.: 91) coding for the full coding region of human ligand polypeptide (SEQ ID NO.: 92).
FIG. 35 shows a comparison of the amino acid sequences in the translation region of bovine ligand polypeptide (SEQ ID NO.: 88) , rat ligand polypeptide (SEQ ID NO.: 90), and human ligand polypeptide (SEQ ID NO.: 92).
FIG. 36 shows the nucleotide sequence of the inserted fragment of plasmid pmGB3 (SEQ ID NO.: 93). The arrowmark indicates the sequence corresponding to the primer.
FIG. 37 shows the cDNA predicted from nucleotide sequence of plasmid pmGB3 (SEQ ID NO.: 93) and the predicted translated protein (SEQ ID NO.: 94). The arrowmark indicates the sequence corresponding to the primer. The sequence between the marks is the sequence predicted to be the intron.
FIG. 38shows the restriction enzyme map of the ligand polypeptide of the present invention.
FIG. 39 shows (i) the nucleotide sequence coding the ligand polypeptide of the present invention and its non-coding region (SEQ ID NO.: 95), and (ii) the amino acid sequence of the ligand polypeptide of the present invention, which obtained in Example 34 (SEQ ID NO.: 96).
FIG. 40 shows the construction figure for the targeting vector pmGFEN28 obtained in Example 35.

FIG. 41 shows the result of the agarose gel electrophoresis described in Example 36, and the comparative gene map between the wild type and the recombinant (knock out) type.!

AB The present invention relates to the murine- derived ligand polypeptide for the G protein-coupled receptor proteins. The ligand polypeptide or the DNA which codes for the ligand polypeptide can be used for (1) development of medicines such as pituitary function modulators, central nervous system function modulators, and pancreatic function modulators, (2) development of receptor binding assay systems using the expression of recombinant receptor proteins and screening of pharmaceutical candidate compounds, and (3) production of nonhuman transgenic animals or non-human knockout animals for analyzing a function of the genes.

CLMN 12 41 Figure(s).

FIG. 1 shows the nucleotide sequence of the human pituitaryderived G protein-coupled receptor protein cDNA fragment harbored in cDNA clone p19P2 isolated by PCR using human pituitary-derived cDNA (SEQ ID NO.:63) and the amino acid (SEQ ID NO.: 64) encoded by the nucleotide sequence. The primer used for sequencing was-21M13. The underscored region corresponds to the synthetic primer.

FIG. 2 shows the nucleotide sequence of the human pituitaryderived G protein-coupled receptor protein cDNA fragment harbored in cDNA clone p19P2 isolated by PCR using human pituitary-derived cDNA (SEQ ID NO.: 65) and the amino acid sequence encoded thereby (SEQ ID NO.: 66). The primer used for sequencing was M13RV-N (Takara). The underscored region corresponds to the synthetic primer.

FIG. 3 shows a partial hydrophobic plot of the protein encoded by the human pituitary-derived G protein-coupled receptor protein cDNA fragment harbored in p19P2 constructed according to the amino acid sequence shown in FIG. 1.

FIG. 4 shows a partial hydrophobic plot of the protein encoded by the human pituitary-derived G protein-coupled receptor protein cDNA fragment harbored in p19P2 constructed according to the amino acid sequence shown in FIG. 2.

FIG. 5 is a diagram comparing the partial amino acid sequence of the protein encoded by the human pituitary-derived G proteincoupled receptor protein cDNA fragment harbored in p19P2 as shown in FIGS. 1 (SEQ ID NO.: 67) and FIG. 2 (SEQ ID NO.: 68), with the known G protein-coupled receptor protein S12863 (SEQ ID NO.: 69). The shadowed region represents the region of agreement. The 1st to 9th amino acid sequence of p19P2 corresponds to the 1st to 99th amino acid sequence of FIG. 1 and the 156th to 230th amino acid sequence corresponds to the 1st to 68th amino acid sequence of FIG. 2.

FIG. 6 shows the nucleotide sequence of the MIN6-derived G protein-coupled receptor protein cDNA fragment based on the nucleotide sequences of the MIN6-derived G protein-coupled receptor protein cDNA fragments harbored in the cDNA clones pG32 and pG1-10 isolated by PCR using MIN6-derived cDNA and the amino acid sequence (SEQ ID NO.: 71) encoded by the nucleotide sequence (SEQ ID NO.: 70). The underscored region corresponds to the synthetic primer.

FIG. 7 is a diagram comparing the partial amino acid sequence encoded by pG3-2/pG1-10 of the MIN6-derived G protein-coupled receptor protein shown in FIG. 6 (SEQ ID NO.: 72) with the partial amino acid sequence of the protein encoded by p19P2 shown in FIGS. 1 and 2. The shadowed region corresponds to the region of agreement. The 1st to 99th amino acid sequence of the protein encoded by p19P2 corresponds to the 1st to 99th amino acid sequence of FIG. 1 and the 156th to 223rd amino acid sequence corresponds to the 1st to 68th amino acid sequence of FIG. 2. The 1st to 223rd amino acid sequence of the protein encoded by pG3-2/pG1-10 corresponds to the 1st to 223rd amino acid sequence of FIG. 6.

FIG. 8 is a partial hydrophobic plot of the MIN6-derived G protein-coupled receptor protein constructed according to the partial amino acid sequence

shown in FIG. 6.

FIG. 9 shows the entire nucleotide sequence of the human pituitary-derived G protein-coupled receptor protein cDNA harbored in the cDNA clone phGR3 isolated from a human pituitary-derived cDNA library by the plaque hybridization method using the DNA fragment inserted in p19P2 as a probe and the amino acid sequence (SEQ ID NO.: 74) encoded by the nucleotide sequence (SEQ ID NO.: 73).

FIG. 10 shows the results of Northern blotting of human pituitary mRNA hybridized with radioisotope-labeled human pituitary cDNA clone phGR3.

FIG. 11 shows a hydrophobic plot of the protein encoded by the human pituitary-derived G protein-coupled receptor protein cDNA harbored in the phGR3 as constructed according to the amino acid sequence shown in FIG. 9.

FIG. 12 shows the nucleotide sequence of the MIN6-derived G protein-coupled receptor protein cDNA fragment (SEQ ID NO.: 75) harbored in the cDNA clone p5S38 isolated by PCR using MIN6derived cDNA and the amino acid sequence (SEQ ID NO.: 76) encoded by the nucleotide sequence. The underscored region corresponds to the synthetic primer.

FIG. 13 shows a diagram comparing the partial amino acid sequence (SEQ ID NO.: 76) of MIN6-derived G protein-coupled receptor protein encoded by p5S38 shown in FIG. 12 with the partial amino acid sequence of G protein-coupled receptor protein encoded by the cDNA fragment harbored in p19P2 as shown in FIGS. 1 and 2 and the partial amino acid sequence of G protein-coupled receptor protein encoded by the nucleotide sequence generated from the nucleotide sequences of cDNA fragments contained in pG3-2 and pG1-10 shown in FIG. 6. The shadowed region represents the sequence region of agreement. The 1st to 144th amino acid sequence of the protein encoded by p5S38 corresponds to the 1st to 144th amino acid sequence of FIG. 12, the 1st to 99th amino acid sequence of the protein encoded by p19P2 corresponds to the 1st to 99th amino acid sequence of FIG. 1 and the 156th to 223rd amino acid sequence corresponds to 1st to 68th amino acid sequence of FIG. 2. The 1st to 223rd amino acid sequence of the protein encoded by pG32/pG1-10 corresponds to the 1st to 223rd amino acid sequence of FIG. 6.

FIG. 14 shows a partial hydrophobic plot of the protein encoded by the MIN6-derived G protein-coupled receptor protein cDNA harbored in p5S38 as constructed according to the partial amino acid sequence shown in FIG. 12.

FIG. 15 shows the results of the following analysis. Thus, RTPCR was carried out to confirm the expression of mRNA in CHO cells transfected by PAKKO-19P2. Lanes 1-7 represent the results obtained by performing PCRs using serial dilutions of pAKKO-19P2 for comparison, i.e. the 10 mu l/ml stock solution (lane 1), 1/2 dilution (lane 2), 1/4 dilution (lane 3), (fraction (1/64)) dilution (Lane 4), (fraction (1/256)) dilution (lane 5), (fraction (1/1024)) dilution (lane 6), and (fraction (1/4096)) dilution (lane 7) of the plasmid as templates, and analyzing the reaction mixtures by 1.2% agarose gel electrophoresis. Lanes 8 through 11 are the results obtained by performing PCRs using a (fraction (1/10)) dilution (lane 8), a (fraction (1/100)) dilution (lane 9), and a (fraction (1/1000)) dilution (lane 10) of the cDNA prepared from the CHO-19P2 cell line as templates and subjecting the respective reaction mixtures to electrophoresis. Lane 11 was obtained by performing PCR using a template obtained by carrying out cDNA synthesis without reverse transcriptase and subjecting the PCR reaction product to electrophoresis. Lanes 12 and 13 were obtained by performing PCR using cDNAs prepared from mock CHO cells with and without addition of reverse transcriptase, respectively, as templates and subjecting the respective reaction products to electrophoresis. M represents the DNA size marker. The lanes at both ends were obtained by electrophoresing 1 mu l of lambda /Sty I digest (Nippon Gene) and the second lane from right was obtained with 1 mu l of theta / chi 174/Hinc II digest (Nippon Gene). The arrowmark indicates the

position of the band amplified by PCR of about 400 bp.

FIG. 16 shows the activity of the crude ligand peptide fraction extracted from rat whole brain to promote release of arachidonic acid metabolites from CHO-19P2 cells. The arachidonic acid metabolite releasing activity was expressed as % of the amount of (3H) arachidonic acid metabolites released in the presence of the crude ligand polypeptide fraction with the amount of (3H) arachidonic acid metabolites released in the presence of 0.05% BAS-HABB being taken as 100%. The activity to promote release of arachidonic acid metabolites from the CHO19P2 cell line was detected in a 30% CH3CN fraction.

FIG. 17 shows the activity of the crude ligand polypeptide fraction extracted from bovine hypothalamus to promote release of arachidonic acid metabolites from CHO-19P2 cells. The arachidonic acid metabolite release-promoting activity was expressed as % of the amount of (3H) arachidonic acid metabolites released in the presence of the crude ligand polypeptide fraction with the amount of (3H) arachidonic acid metabolites released in the presence of 0.05% BAS-HABB being taken as 100%. The activity to promote release of arachidonic acid metabolites from the CHO-19P2 cell line was detected in a 30% CH3CN fraction just as in the crude ligand polypeptide fraction from rat whole brain.

FIG. 18 shows the activity of the fraction purified with the reversed-phase column C18 218TP5415 to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells. The active fraction from RESOURCE S was fractionated on C18 218TP5415. Thus, chromatography was carried out at a flow rate of 1 ml/min. on a concentration gradient of 20%-30% CH3CN/0.1% TFA/H2O, the eluate was collected in 1 ml fractions, and each fraction was lyophilized. Then, the activity of each fraction to specifically promote release of arachidonic acid metabolites from the CHO-19P2 cell line was determined. As a result, the activity was fractionated into 3 fractions (designated, in the order of elution, as P-1, P-2, and P-3).

FIG. 19 shows the activity of the fraction purified with the diphenyl 219TP5415 reversed-phase column to specifically promote arachidonic acid metabolite release from CHO-19P2 cells. The P-3 active fraction from C18 218TP5415 was fractionated on diphenyl 219TP5415. The chromatography was carried out at a flow rate of 1 ml/min. on a concentration gradient of 22%-25% CH3CN/0.1% TFA/H2O, the eluate was collected in 1 ml fractions, and each fraction was lyophilized. Then, the activity to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells in each fraction was determined. As a result, the activity converged in a single peak.

FIG. 20 shows the activity of the fraction purified by mu RPC C2/ C18 SC 2.1/10 reversed-phase column to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells. The peak active fraction from diphenyl 219TP5415 was fractionated on mu RPC C2/C18 SC 2.1/10. The chromatography was carried out at a flow rate of 100 mu l/min. on a concentration gradient of 22%-23.5% CH3CN/0.1% TFA/H2O, the eluate was collected in 100 mu l fractions, and each fraction was lyophilized. Then, the activity to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells in each fraction was determined. As a result, the activity was found as two peaks of apparently a single substance (peptide).

FIG. 21 shows the activity of the P-2 fraction purified by mu RPC C2/C18 SC 2.1/10 reversed-phase column to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells. The chromatography was carried out at a flow rate of 100 mu l/min. on a concentration gradient of 21.5%-23.0% CH3CN/0.1% TFA/dH2O, the eluate was collected in 100 mu l fractions, and each fraction was lyophilized. Then, the activity to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells in each fraction was determined. As a result, the activity was found as a peak of

apparently a single substance.

FIG. 22 shows the nucleotide sequence of bovine hypothalamus ligand polypeptide cDNA fragment (SEQ ID NO.: 77) as derived from the nucleotide sequence of the bovine hypothalamus-derived ligand polypeptide cDNA fragment which specifically promotes release of arachidonic acid metabolites from CHO-19P2 cells as harbored in a cDNA clone isolated by PCR using bovine hypothalamus-derived cDNA and the amino acid sequence (SEQ ID NO.: 78) encoded by said nucleotide sequence. The region indicated by the arrowmark corresponds to the synthetic primer.

FIG. 23 shows the nucleotide sequence of the bovine hypothalamusderived ligand polypeptide cDNA fragment (SEQ ID NO.: 79) generated according to the nucleotide sequence of the bovine hypothalamus-derived ligand polypeptide cDNA fragment which specifically promotes release of arachidonic acid metabolites from CHO-19P2 cells as harbored in a cDNA clone isolated by PCR using bovine hypothalamus-derived cDNA and the amino acid sequence (SEQ ID NO.: 80) encoded by said nucleotide sequence. The region indicated by the arrowmark corresponds to the synthetic primer.

FIG. 24 shows the amino acid sequences of the bovine hypothalamus-derived ligand polypeptides which specifically promote release of arachidonic acid metabolites from CHO-19P2 cells; FIG. 24a (SEQ ID NO.: 82), FIG. 24b (SEQ ID NO.: 84), and the cDNA sequence coding for the full coding region of the ligand polypeptides defined by SEQ ID NO.: 1 and SEQ ID NO.: 44; FIG. 24a (SEQ ID NO.: 81), FIG. 24b (SEQ ID NO.: 83).

FIG. 25 shows the concentration-dependent activity of synthetic ligand polypeptide (19P2-L31) to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells. The synthetic peptide was dissolved in degassed d H2O at a final concentration of 10-3M and diluted with 0.05% BSA-HBSS to concentrations of 10-12M-10-6M. The arachidonic acid metabolite releasing activity was expressed in the measured radioactivity of (3H) arachidonic acid metabolites released in the supernatant when the dilution was added to the cells. As a result, the activity of 19P2-31 to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells was found in a concentration-dependent manner.

FIG. 26 shows the concentration-dependent activity of synthetic ligand polypeptide (19P2-L31(O)) to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells. The synthetic ligand peptide was dissolved in degassed d H2O at a final concentration of 10-3M and diluted with 0.05% BSA-HBSS to concentrations of 10-12M-10-6M. The arachidonic acid metabolite releasing activity was expressed in the measured radioactivity of (3H) arachidonic acid metabolites released in the supernatant when the dilution was added to the cells. As a result, the activity of 19P2-L31(O) to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells was found in a dose-dependent manner.

FIG. 27 shows the activity of synthetic ligand polypeptide 19P2L20 to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells. The synthetic peptide was dissolved in degassed distilled water at a final concentration of 10-3M and diluted with 0.05% BSA-HBSS to concentrations of 10-12M-10-6M. The arachidonic acid metabolite releasing activity was expressed in the measured radioactivity of (3H) arachidonic acid metabolites released in the supernatant when the dilution was added to the cells. As a result, the activity of 19P2-L20 to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells was found in a dose-dependent manner.

FIG. 28 shows the 1.2% agarose gel electrophoregram of the DNA fragments of the phages cloned from a bovine genomic library as digested with restriction enzymes BamHI(B) and SalI(S). As the DNA size marker (M), StyI digests of lambda phage DNA were used. In lane B, two bands derived

from the vector were detected in positions between the first (19,329 bp) and second (7.743 bp) marker bands, as well as 3 bands derived from the inserted fragment between the third (6,223 bp) and 5th (3,472 bp) bands. In lane S, two bands derived from the vector were similarly detected but due to the overlap of the band of the inserted fragment, the upper band is thicker than the band in lane B.

FIG. 29 shows the nucleotide sequence around the coding region as decoded from bovine genomic DNA (SEQ ID NO.: 85). The 1st to 3rd bases (ATG) correspond to the translation start codon and the 767th to 769th bases (TAA) correspond to the translation end codon.

FIG. 30 shows a comparison between the nucleotide sequence (genome) around the coding region as deduced from bovine genomic DNA (SEQ ID NO.: 85) and the nucleotide sequence (cDNA) of bovine cDNA cloned by PCR (SEQ ID NO. :87). The sequence region of agreement is indicated by shading. As to the 101st to 572nd region, there is no corresponding region in the nucleotide sequence of cDNA, indicating that it is an intron.

FIG. 31 shows the translation of the amino acid sequence (SEQ ID NO.: 88) encoded after elimination of the intron from the nucleotide sequence around the coding region as decoded from bovine genomic DNA (SEQ ID NO.: 87).

FIG. 32 shows the full-length amino acid sequence (SEQ ID NO.: 90) and the cDNA sequence coding for the full coding region of rat ligand polypeptide (SEQ ID NO.:89).

FIG. 33 shows the amino acid sequence of bovine ligand polypeptide (SEQ ID NO.: 88) and the nucleotide sequences of DNAs coding for bovine polypeptide (SEQ ID NO.: 87) and rat polypeptide (SEQ ID NO.: 89). The arrowmark indicates the region corresponding to the synthetic primer.

FIG. 34 shows the full-length amino acid sequence and the sequence of cDNA (SEQ ID NO.: 91) coding for the full coding region of human ligand polypeptide (SEQ ID NO.: 92).

FIG. 35 shows a comparison of the amino acid sequences in the translation region of bovine ligand polypeptide (SEQ ID NO.: 88) , rat ligand polypeptide (SEQ ID NO.: 90), and human ligand polypeptide (SEQ ID NO.: 92).

FIG. 36 shows the nucleotide sequence of the inserted fragment of plasmid pmGB3 (SEQ ID NO.: 93). The arrowmark indicates the sequence corresponding to the primer.

FIG. 37 shows the cDNA predicted from nucleotide sequence of plasmid pmGB3 (SEQ ID NO.: 93) and the predicted translated protein (SEQ ID NO.: 94). The arrowmark indicates the sequence corresponding to the primer. The sequence between the marks is the sequence predicted to be the intron.

FIG. 38shows the restriction enzyme map of the ligand polypeptide of the present invention.

FIG. 39 shows (i) the nucleotide sequence coding the ligand polypeptide of the present invention and its non-coding region (SEQ ID NO.: 95), and (ii) the amino acid sequence of the ligand polypeptide of the present invention, which obtained in Example 34 (SEQ ID NO.: 96).

FIG. 40 shows the construction figure for the targeting vector pmGFEN28 obtained in Example 35.

FIG. 41 shows the result of the agarose gel electrophoresis described in Example 36, and the comparative gene map between the wide type and the recombinant (knock out) type.!

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TITLE: Vectors based on recombinant defective viral genomes, and their use in the formulation of vaccines; porcine vaccine comprises a helper virus and a recombinant virion that has at least 1.9 kb of the 5' end of a TGEV genome, pseudoknot region, including the region of overlap between ORFs 1a and 1b, defective or

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                         missing S, M, and N structural genes, and at least bp
                         9691-9707 of the 3' end of the genome
INVENTOR(S):             Zunzunegui; Ana Mendez, Pontevedra, ES
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                            NUMBER          PK    DATE
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PATENT INFORMATION:      US 7041300         B1  20060509
                         WO 97034008            19970918
APPLICATION INFORMATION: US 1997-155003         19970312
                         WO 1997-ES59           19970312
                                                19980914  PCT 371 date
                                                19980914  PCT 102(e) date
EXPIRATION DATE:         12 Mar 2017

                            NUMBER                DATE
                         ---------------        --------
PRIORITY APPLN. INFO.:   ES 1996-620             19960314
FAMILY INFORMATION:      US 7041300             20060509
DOCUMENT TYPE:           Utility
                         Granted Patent - Utility, no Pre-Grant Publication
                         Certificate of Correction
CORRECTION DATE:         28 Nov 2006
FILE SEGMENT:            CHEMICAL
                         GRANTED
ENTRY DATE:              Entered STN: 10 May 2006
                         Last Updated on STN: 16 Jan 2007

MICROFILM REEL NO:       011243   FRAME NO: 0893
NUMBER OF CLAIMS:        16
GRAPHICS INFORMATION:    27 Drawing Sheet(s),  27 Figure(s).
                         DESCRIPTION OF FIGURES:
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FIG. 1 shows the structure of TGEV. The virion is a spherical particle consisting of a lipidic envelope in whose interior is a single-chain, positive-polarity RNA molecule of 28.5 kilobases (kb). This RNA is associated to an N protein forming the nucleocapsid. M and sM structural proteins are included in the membrane. Protein S groups itself into trimers, and is anchored on the external part of the envelope forming the peplomers.
FIG. 2 shows the genomic organization of the four sequenced coronaviruses: is MHV, IBV, HCV229E (human coronavirus 229E) and TGEV. The open reading frames coding for each protein are shown to scale. In each genome, the beginning of the RNAs expressing each virus is indicated with an arrow. The number of messenger RNAs (mRNA) expressed by viruses MHV or TGEV may vary depending on the viral strain. In this outline, the TGEV arrows correspond to the mRNAs

expressed by strain THER-1. The mRNAs are 3'-coterminal and are numbered in decreasing size order.

FIG. 3 shows the expression of the TGEV genome, strain THER-1. The disposition of the open reading frames (ORF) in the genome is indicated: Pol, polymerase; S, sM, M and N, structural proteins; nsp 3a, 3b and 7, non-structural proteins (protein 3b is not produced with this virus). The genome is transcribed in an RNA of equal length but of negative polarity (-) that will serve as a template for the synthesis of the 7 mRNAs (1 to 7). In each mRNA are represented the common sequence, leader, of the 5' end (square), the polyadenine section at the 3' end and the zone which is translated in each one of them (thick lines).

FIG. 4 shows the evolution of the titer of TGEV THER-1 (A) and PUR46-mar 1CC12 (B) isolates with the passage number at high multiplicity of infection (m.o.i.) in ST cells.

FIG. 5 shows the results of the electrophoretic analysis of the RNAs produced in ST cells infected with THER-1 virus passaged 46 times at high m.o.i. The passage number is indicated above each lane and the bars to the left indicate the position of the molecular weight markers (genomic RNA of the TGEV and GibcoBRL markers), expressed in kb. The bars to the right indicate the TGEV mRNAs and the defective interfering (DI) RNAs. NI, not infected.

FIG. 6 shows the results of the Northern-blot assay of the RNA of ST ***cells*** infected with the THER-1p35 virus.

FIG. 7 shows the results of the Northern-blot assay of the RNA proceeding from diluted passages of the THER-1-STp41 virus in ST cells.

FIG. 8 shows the effect of the cell line change on the propagation of defective RNAs A, B and C. Virus THER-1-STp46 was passaged ten times undiluted in IPEC (intestinal pig epithelium cells), and five times in PM (porcine macrophage) cells. FIGS. 8A and 8B: evolution of the virus titer with the number of the passage in IPEC and PM, respectively. FIG. 8C: analysis of the RNA of ST cells infected with virus from passages 1 and 10 in IPEC (by metabolic labeling with 32PI), or of passages 1 and 5 in PM cells (by hybridization with one oligonucleotide complementary to the leading RNA).

FIG. 9 shows the encapsidation of defective genomes A, B and C. FIG. 9A shows an agarose gel stained with ethidium bromide, in which the RNA extracted from purified virions of passage 1 and passage 41 are analyzed by means of centrifugation through a 15% sucrose cushion (w/v). In the lane of passage 41, RNAs A, B and C can be observed, as well as the parental genome. The bars on the left indicate mobility markers in kb. FIG. 9B shows the results of the analysis of the RNA of passage 41 virions purified by centrifugation through sucrose cushions or continuous gradient, by Northern-blot assay with an oligonucleotide complementary to the leader RNA. Commercial GibcoBRL RNAs and RNA from passage 1 virions (lane a) were used as markers. Lanes b and c, RNA extracted from virions sedimented through 31% and 15% sucrose cushions (w/v), respectively. Lanes d and e, RNA extracted from virus purified through a continuous sucrose cushion, fractions of 1.20 and 1.15 g/ml density, respectively.

FIG. 10 shows the strategy for the cloning of defective RNAs DI-B and DI-C, in which a schematic representation can be observed of the complementary DNA fragments (cDNA) obtained by RT-PCR, using as template genomic RNA of total length (A), DI-B (B) and DI-C (C). The dotted lines indicate absence of the anticipated fragment due to its large size. The defective RNAs were cloned into four overlapping fragments (a, b, c and d), represented by lines; the numbers below these lines indicate fragment size determined in agarose gels. The oligonucleotides used as primers and their polarity are indicated by means of arrows and numbers. Oligonucleotide sequence is shown in Table 2. Striped or open boxes in (A) indicate the relative position of the viral genes: pol, polymerase; S, M and N, structural genes; 3a, 3b, sM and 7, small ORFs. The shaded thin boxes indicate the leader sequence.

FIG. 11 shows the results of the electrophoretic analysis of PCR products obtained in the amplification of defective RNAs. The RNA of purified virions THER-1p1 or THER-1p41 was used as a template in an RT-PCR reaction with

oligonucleotides 1 and 2 (a), 3 and 4 (b), 5 and 6 (c), or 7 and 8 (d), whose sequence and position in the parental genome is indicated in Table 2. The lane corresponding to the RNA template of passage 1 (parental genomic RNA) or of passage 41 (parental RNA, DI-A, DI-B and DI-C), and the lane of DNA mobility markers (M, GibcoBRL) are indicated in each case. The numbers in bold type indicate the size in kb of the amplification products specific for defective RNAs.

FIG. 12 (SEQ ID NOS: 24-27) shows the complete RNA DI-C cDNA sequence (See SEQ ID NO: 24) obtained by the sequencing of overlapping fragments of the a, b, c, and d cloning. RNA DI-C has kept four discontinuous parental genome regions: I, II, III and IV. The flanking sites of these regions are indicated with arrows. The translation of the three ORFs present in genome DI-C is indicated: chimeric ORF of 6.7 kb resulting from the fusion of discontinuous regions I and II in phase; the mini-ORF of three amino acids preceding it in phase; and the ORF, which initiates at the AUG of gene S. Highly homologous regions with the proteic domains described for other coronaviruses as those responsible for the polymerase and helicase activities, and metal ion binding sites appear shaded. CTAAAC transcription promoter sequences appear shaded. The overlapping area between ORFs 1a and 1b (41 nucleotides) appears shaded, the slippery sequence of the ribosome is underlined, and the ORF1a termination codon is in a box. In positions 637, 6397, and 6485, the specific changes with respect to the parental genome are indicated. The nucleotides present in the parental genome in these positions are indicated.

FIG. 13 shows a diagram of the RNA DI-C structure. Total genomic length appears to the right of the boxes. RNA DI-C contains four discontinuous TGEV genome regions (I, II, III and IV). These regions comprise 2.1 kb of the 5' end of the genome, almost complete ORF1b including the overlapping area between ORFs 1a and 1b, the initiation of gene S, incomplete ORF7 and untranslated region 3'. The letters and numbers above the parental genome box indicate viral genes. The numbers below the box indicate the position of the flanking nucleotides of the discontinuous regions in the parental genome, taking as reference the sequence of the TGEV PUR46-PAR isolate. In the box corresponding to RNA DI-C, the length of the four discontinuous regions is indicated in nucleotides. In the third box it is indicated the number of nucleotides derived from each viral gene, taking into account that ORFs 1a and 1b overlap with each other 43 nucleotides in the parental genome. The ORFs predicted in the computer analysis are indicated with arrows or arrowheads. Pnt, pseudoknot; Pol, polymerase; Mib, metal ion binding; Hel, helicase; Cd, conserved domain.

FIG. 14 shows the structure of the RNA DI-B. RNA DI-B contains three discontinuous regions (I, II and III) of the TGEV genome, comprising, 2.1 kb of the 5' end of the genome, complete ORF1b including the overlapping area between ORFs 1a and o 1b, the initiation of gene S, the termination of ORF7, and the untranslated region of 3' end. Letters and numbers over the parental genome box indicate viral genes. The numbers underneath the box indicate the position in the parental genome of the flanking nucleotides of the discontinuous regions, taking as reference the sequence of the TGEV PUR46-PAR isolate. Size heterogeneity of the deletion occurring between discontinuous regions II and III is responsible for the actual existence of a DI-B genome population. In the second and third boxes are indicated the length in nucleotides of the three discontinuous regions for the largest and smallest sized genomes, respectively. In the third box it is indicated the number of nucleotides derived from each viral gene, taking into account that ORFs 1a and 1b overlap each other for 43 nucleotides in the parental genome. The ORFs predicted by the computer analysis are indicated with arrows or arrowheads. Pnt, pseudoknot; Pol, polymerase; Mib, metal ion binding; Hel, helicase; Cd, conserved domain.

DESCRIPTION OF FIGURES:

FIG. 15 shows the secondary and tertiary RNA structures in the overlapping zone between ORFs 1a and 1b in the RNA DI-C. (A) (SEQ ID NO: 28) Structure predicted when considering the region closest to the fork-like structure presenting complementarity to the nucleotides of the knot thus constituting the pseudoknot (nucleotides 2354 to 2358). The slippery sequence UUUAAAC is underlined. ORF1a

termination codon is indicated in the box. (B) Schematic representation of this pseudoknot, which involves two sequence complementarity sections (stems: S1 and S2). The slippery sequence is represented in a box. (C) (SEQ ID NO: 29 and SEQ ID NO: 30) An alternative model taking into account the sequence of nucleotide 2489 to 2493 in the folding of the pseudoknot. This structure contains an additional complementarity sequence (stem) section. (D) Schematic representation of the pseudoknot, in which the three stems are marked: S1, S2 and S3.

FIG. 16 shows the mapping of RNAs DI by hybridization with oligonucleotides specific to the virus in Northern-blot assays. The RNA of THER-1-STp41 virus was fractionated in agarose gels until a clear separation of the parental genome RNAs and DI A, B and C was obtained. The RNA was transferred to nylon filters which were hybridized with several oligonucleotides labeled with 32Pi, hybridized with the parental genome (+), and hybridized (+) or not (-) with the defective genomes. The approximate localization of the sequences complementary to the oligonucleotides in the parental genome is indicated by arrows. Their exact sequence and position are indicated in Table 3. All the oligonucleotides hybridized with the parental genome and gave the expected results with RNAs B and C.

FIG. 17 shows an outline of the method for the obtainment of vaccinal viruses by transfection of infected cells with RNA DI-C. A prototype outline is illustrated with the construction that enabled the production of DI-C RNA by in vitro transcription, maintaining the 5' and 3' ends present in the original defective particle. The sequence of the T7 promoter [PrT7] (SEQ ID NO: 31) and the sequence of the autocatalytic ribozyme of the hepatitis delta virus (HDV) [Rz HDV] (SEQ ID NO: 32) were cloned flanking the DI-C RNA sequence. The position of the autocatalytic cleavage introduced by the ribozyme is marked above the sequence. The arrows indicate the positions of the internal transcription promoter sequences maintained in a natural form in the RNA DI-C. L: leader. T7 phi : T7 bacteriophage transcription termination signals. Virions encapsidating both the helper virus as well as the defective genomes in which the heterologous genes had been cloned were recovered, when the in vitro transcribed RNAs were transfected into ST cells infected with the corresponding helper virus.

FIG. 18 shows a prototype outline of the construction that enabled the production of pDIA-6A.C3 by in vitro transcription, maintaining the 3' and 5' ends present in the original defective particle. The sequence of the bacteriophage T7 promoter [PrT7] and the presence of the autocatalytic ribozyme of the hepatitis delta virus (HDV) [RzHDV] were cloned flanking the cDNA sequence coding for an autoreplicative RNA. Plasmid pDIA-6A.C3 contains the gene coding for monoclonal antibody 6A.C3 which neutralizes TGEV [see Example 4]. The cloning of the heterologous gene was done after ORF1b, following the S gene initiation codon (AUG), and in reading phase with this gene (IGS: intergenic sequence; L leader sequence; D: diversity region; J: joining region; VH: variable module of the immunoglobulin heavy chain; CH: constant module of the immunoglobulin heavy chain; VK: variable module of the immunoglobulin light chain; CK: constant module of the immunoglobulin light chain; polyA: polyA sequence; T7 phi : 17 transcription terminator].!

AB The vectors comprise a recombinant defective viral genome expressing at least one antigen suitable for the induction of systemic and secretory immune responses or an antibody conferring protection against an infectious agent. The defective viral genome comprises the genome of a parental virus having the viral replicase recognition signals located on ends 3' and 5', further comprising internal deletions, and wherein said defective viral genome depends on a helper virus for its replication and encapsidation. These vectors are suitable for the forming of a recombinant system comprising the aforesaid expression vector, and a helper virus. The system is suitable for the manufacture of mono- and polyvalent vaccines against infectious agents of different animal species, especially pigs, dogs and cats, and as expression vehicles for antibodies protective against infectious agents.

CLMN 16
GI 27 Drawing Sheet(s), 27 Figure(s).

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L7 ANSWER 20 OF 33 CABA COPYRIGHT 2009 CABI on STN

ACCESSION NUMBER: 2001:21922 CABA <<LOGINID::20090224>>
DOCUMENT NUMBER: 20003017660
TITLE: Biochemical and immunological properties of a viral hybrid particle expressing the Plasmodium vivax merozoite surface protein 1 C-terminal region
AUTHOR: Wunderlich, G.; Portillo, H. A. del; del Portillo, H. A.
CORPORATE SOURCE: Departamento de Parasitologia, Instituto Ciencias Biomedicas II, Universidade de Sao Paulo, Sao Paulo 05508-900, SP, Brazil.
SOURCE: Molecular Medicine, (2000) Vol. 6, No. 3, pp. 238-245. 26 ref.
Publisher: Johns Hopkins University Press. Baltimore
ISSN: 1076-1551
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal
LANGUAGE: English
ENTRY DATE: Entered STN: 2 Mar 2001
Last Updated on STN: 2 Mar 2001

AB Mammalian cells expressing the small hepatitis B virus surface protein (HBs) secrete highly immunogenic 20 nm lipoprotein particles. Previous studies demonstrated that the fusion of foreign sequences into certain regions of HBs leads to chimaeric particles carrying epitopes for the foreign peptide, as well as for HBs. This study investigated the immunological and biochemical properties of the fusion of the C-terminal region of the merozoite surface protein 1 of Plasmodium vivax and HBs (PvMSP119-HBs). COS7 cells were transfected with a plasmid coding for PvMSP119-HBs. The hybrid products were analysed by density gradient centrifugation and electron microscopy or detected by metabolic labelling and immunoprecipitation with anti-HBs and patient-derived anti-P. vivax serum. Mice were immunized with the vector and the antibody response was checked by ELISA. The fusion PvMSP119-HBs formed particles of 20-45 nm size which were secreted from COS7 cells. The particles were immunoprecipitable with anti-HBs and serum of different P. vivax-positive individuals. Immunization of mice with the construct as a genetic vaccine showed that antibodies were raised mostly against the PvMSP119 domain and recognized the native protein. Due to its biochemical and antigenic properties, the hybrid particle will be useful in future vaccine trials against asexual blood stages of P. vivax as a genetic and/or a proteic subunit candidate.

L7 ANSWER 21 OF 33 CABA COPYRIGHT 2009 CABI on STN

ACCESSION NUMBER: 92:67115 CABA <<LOGINID::20090224>>
DOCUMENT NUMBER: 19921446475
TITLE: Ruminant hepatic metabolism of volatile fatty acids, lactate and pyruvate

AUTHOR: Armentano, L. E.
CORPORATE SOURCE: 1675 Observatory Drive, Madison, WI 53706, USA.
SOURCE: Journal of Nutrition, (1992) Vol. 122, No. 3S, pp. 838-842. 34 ref.
ISSN: 0022-3166
DOCUMENT TYPE: Journal
LANGUAGE: English
ENTRY DATE: Entered STN: 1 Nov 1994
Last Updated on STN: 1 Nov 1994

AB Ruminant liver has a quantitatively unique array of substrates presented to it because of the extensive fermentation of dietary carbohydrate to organic acids in the gastrointestinal tract. The single largest input of dietary energy to the extrasplanchnic tissues is acetic acid derived from fermentation, which is largely unused by hepatic parenchyma. The other volatile fatty acids derived from fermentation, primarily propionate, are cleared extensively, but not completely, by the liver. This results in a marked concentration gradient for these acids across the liver lobule. L-Lactate, derived from tissue metabolism , as well as variable amounts from rumen fermentation, is used by the liver at a rate lower than for propionate and below the predicted capacity based on enzymic and intact cell capacity data obtained in vitro. The net result of this selective utilization by the liver results in peripheral blood containing significant concentrations of L-lactate and acetate, but little of the other organic acids. Propionate carbon metabolized by liver cells is converted to glucose with little true loss of carbon, but the same is not true of lactate carbon. The energetic efficiencies by which propionate and lactate carbon are converted to glucose may be much less than optimum because of extensive cycling through pyruvate kinase, pyruvate carboxylase and phosphoenolpyruvate carboxykinase. Inhibition of this futile cycling may represent one avenue by which energetic costs of maintenance and production can be lowered in ruminants.

L7 ANSWER 22 OF 33 CABA COPYRIGHT 2009 CABI on STN
ACCESSION NUMBER: 88:94481 CABA <<LOGINID::20090224>>
DOCUMENT NUMBER: 19881416713
TITLE: Potassium, sodium and magnesium requirements of grazing ruminants
AUTHOR: Beringer, H.
CORPORATE SOURCE: Agricultural Research Station Buntehof, Hannover, German Federal Republic.
SOURCE: Potash Review, (1988) No. 2, pp. 10. 21 ref.
ISSN: 0032-5546
DOCUMENT TYPE: Journal
LANGUAGE: English
ENTRY DATE: Entered STN: 1 Nov 1994
Last Updated on STN: 1 Nov 1994

AB The need for cheap, high quality farm-produced fodder for milk production, herd fertility and health is discussed. K^+ and Mg^{++} are essential for many enzyme reactions and both are found in high concentration within animal cells, whereas Na^+ and Cl^- dominate in the blood and extracellular liquids. This ionic distribution is the basis of electrochemical gradients involved in energy-requiring metabolic processes and in the transmission of signals in sensory, nerve and muscle cells. The animal has efficient control and hormonal response mechanisms to maintain electrolytic balance. Aldosterone, a hormone of the adrenal gland, is most important in Na homeostasis. A grass diet does not generally satisfy the Mg and Na needs of grazing cows (30 g/30 litres milk daily). Under conditions in which the K/Na ratio in feed exceeds 30:1 and saliva Na concentration falls below 130 mM Na, Mg resorption is impaired

and decreasing herd fertility (low conception rate) is likely. Mg and Na can be offered as salt licks, but acceptance by individual animals is variable. Pastures should contain a high proportion of species (especially Lolium perenne) which take up Na and respond well to fertilizers containing Na and Mg.

L7 ANSWER 23 OF 33 BIOTECHNO COPYRIGHT 2009 Elsevier Science B.V. on STN

ACCESSION NUMBER: 1995:25065022 BIOTECHNO <<LOGINID::20090224>>

TITLE: Effect of physiological ADP concentrations on contraction of single skinned fibers from rabbit fast and slow muscles

AUTHOR: Chase P.B.; Kushmerick M.J.

CORPORATE SOURCE: Dept. of Radiology, University of Washington,Seattle, WA 98195, United States.

SOURCE: American Journal of Physiology - Cell Physiology, (1995), 268/2 37-2 (C480-C489)
CODEN: AJPCDD ISSN: 0363-6143

DOCUMENT TYPE: Journal; Article

COUNTRY: United States

LANGUAGE: English

SUMMARY LANGUAGE: English

AN 1995:25065022 BIOTECHNO <<LOGINID::20090224>>

AB To directly assess the possible role of ADP in muscle fatigue, we have studied the effect of physiological MgADP levels on maximum Ca.sup.2.sup.+-activated isometric force and unloaded shortening velocity (V(us)) of single skinned fiber segments from rabbit fast-twitch (psoas) and slow-twitch (soleus) muscles. MgADP concentration was changed in a controlled and well-buffered manner by varying creatine (Cr) in solutions, which also contained MgATP, phosphocreatine (PCr), and creatine kinase (CK). To quantify ADP as a function of Cr added, we determined the apparent equilibrium constant (K') of CK for the conditions of our experiments (pH 7.1, 3 mM Mg.sup.2.sup.+, 12°C): K' = (Σ¢Cr!-Σ¢ATP!)/(Σ¢PCr! .midldot. Σ¢ADP!) = 260 ± 3 (SE). In this manner, ADP was altered essentially as occurs during stimulation in vivo but without the concomitant changes in pH and P(i), which affect force and V(us). As ADP (and Cr) was increased, force and V(us) decreased in both fiber types; at the highest ADP level used, 200 μM, normalized force was 96.6 ± 1.7% for psoas (n = 6) and 93.7 ± 2.8% for soleus (n = 6), and V(us) was 80.4 ± 2.4% for psoas and 91.3 ± 7.7% for soleus. Diffusion-reaction on calculations indicated that radial gradients of metabolite concentrations within fibers could not explain the small effects of ADP on fiber mechanics, and experiments verified that metabolite levels were well buffered within fibers by the CK reaction. Exogenous CK was added to bathing solutions at 290 U/ml, threefold above that necessary to maintain V(us) independent of CK concentration; in the absence of PCr and exogenous CK, at least a fourfold increased MgATP was necessary to maintain V(us) at the control level. Adenylate kinase activity was not detectable; thus myofibrillar adenosine- triphosphatase and exogenous CK activities were the major determinants of nucleotide levels within activated cells. Cr alone (in absence of PCr and exogenous CK) also decreased force and V(us), presumably by a nonspecific mechanism. Over the physiological range, altered ADP had little or no effect on force or V(us) in well-buffered conditions. It is therefore likely that other factors decrease force and V(us) during muscular fatigue.

L7 ANSWER 24 OF 33 BIOTECHNO COPYRIGHT 2009 Elsevier Science B.V. on STN

ACCESSION NUMBER: 1989:20009792 BIOTECHNO <<LOGINID::20090224>>

TITLE: Regulation of pH in individual pancreatic β-cells

as evaluated by fluorescence ratio microscopy

AUTHOR: Grapengiesser E.; Gylfe E.; Hellman B.

CORPORATE SOURCE: Dept. of Medical Cell Biology, Biomedicum, University of Uppsala Box 571,S-75123 Uppsala, Sweden.

SOURCE: Biochimica et Biophysica Acta - Molecular Cell Research, (1989), 1014/3 (219-224)
CODEN: BAMRDP ISSN: 0167-4889

DOCUMENT TYPE: Journal; Article

COUNTRY: Netherlands

LANGUAGE: English

SUMMARY LANGUAGE: English

AN 1989:20009792 BIOTECHNO <<LOGINID::20090224>>

AB Pancreatic β- cells are known to maintain intracellular pH (pH(i)) at a value well above that predicted from the electrochemical gradient. The mechanisms for the active extrusion of protons were examined by continuously monitoring pH(i) in individual β- cells from ob/ob mice using the fluorescent indicator 2',7'-bis(carboxyethyl)-5(6)-carboxyfluorescein (BCECF). In a medium nominally devoid of bicarbonate, the steady-state pH(i) was 6.82 ± 0.02 and the intracellular buffering capacity was equivalent to 79 ± 3 mM/pH unit. pH(i) remained unaffected after raising the glucose concentration from 3 to 20 mM, it was lowered when depolarizing the β- cells with tolbutamide and it increased in the presence of carbachol. After removal of Na.sup.+ there was a significant drop of pH(i) and blockage of the pH(i) recovery following acid loading with the NH.sub.4.sup.+ prepulse technique. Whereas addition of amiloride had a similar, but less pronounced effect, omission of Cl.sup.- resulted in moderate alkalinisation. After switching to a medium containing bicarbonate, minor acidification was followed by adjustment of pH(i) to a steady state higher than the initial one. The results indicate that the acid load arising from glucose metabolism in the β- cells is effectively buffered and the protons extruded both by Na.sup.+-H.sup.+ and Cl.sup.--HCO.sub.3.sup.- exchangers.

L7 ANSWER 25 OF 33 BIOTECHNO COPYRIGHT 2009 Elsevier Science B.V. on STN

ACCESSION NUMBER: 1989:19255496 BIOTECHNO <<LOGINID::20090224>>

TITLE: Progesterone production from granulosa cells of individual human follicles derived from diabetic and nondiabetic subjects

AUTHOR: Diamond M.P.; Lavy G.; Lake Polan M.

CORPORATE SOURCE: Department of Obstetrics and Gynecology, Yale University School of Medicine, New Haven, CT 06510, United States.

SOURCE: International Journal of Fertility, (1989), 34/3 (204-208)
CODEN: INJFA3 ISSN: 0020-725X

DOCUMENT TYPE: Journal; Article

COUNTRY: United States

LANGUAGE: English

SUMMARY LANGUAGE: English

AN 1989:19255496 BIOTECHNO <<LOGINID::20090224>>

AB Insulin and insulin-like growth factors have been implicated in the stimulation of ovarian steroidogenesis. To assess the effect of diabetes mellitus on this process, a comparison was made of progesterone production by cultured granulosa cells (50,000 cells/ well) from 11 individual follicles of nondiabetic and 6 individual follicles of diabetic women. Diabetic metabolic control was fair ¢HbA.sub.1C 6.8, 8.7 (nl 5.0-7.5)!. Cells were collected by laparoscopic follicular aspiration after ovulation induction and isolated by Percoll gradient centrifugation. Progesterone production was measured

after culture with hCG (10 IU/mL) or insulin (100 μU/mL). In both nondiabetic and diabetic groups on day 4, hCG significantly stimulated progesterone production (1,686 ± 1,268 ng/mL to 4.123 ± 2,825 ng/mL and 1,059 ± 249 ng/mL to 1,506 ± 245 ng/mL respectively). In nondiabetic follicles, insulin also stimulated progesterone production on days 4 (2366 ± 1032 ng/mL to 3699 ± 1582 ng/mL; $P < .05$) and 7 (987 ± 475 ng/mL to 1858 ± 929 ng/mL; $P < .05$); this response was not noted in diabetic granulosa cells. We suggest that insulin-stimulated progesterone production by granulosa cells isolated in the presence of fair diabetic metabolic control is impaired.

L7 ANSWER 26 OF 33 BIOTECHNO

ACCESSION NUMBER: 1986:16008308 BIOTECHNO <<LOGINID::20090224>>

TITLE: Oxygenation and differentiation in multicellular spheroids of human colon carcinoma

AUTHOR: Sutherland R.M.; Sordat B.; Bamat J.; et al.
CORPORATE SOURCE: Department of Radiation Biology, University of Rochester Cancer Center, Rochester, NY, United States.

SOURCE: Cancer Research, (1986), 46/10 (5320-5329)
CODEN: CNREA8
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
AN 1986:16008308 BIOTECHNO <<LOGINID::20090224>>

AB Oxygenation and development of necrosis were evaluated in multicellular spheroids of poorly differentiated (HT29) and moderately well -differentiated (Co112) human adenocarcinoma of the colon. Spheroids were grown in vitro under well-controlled oxygen and nutrient conditions in spinner flasks up to sizes of 2800-μm diameter after 5 wk of culture. Morphological studies showed that the Co112 spheroids contained pseudoglandular structures with lumen, very similar to the characteristics of the original tumor specimen from the patient and to the cells when grown as xenograft tumors in nude mice. Microelectrodes were used to measure the oxygen tension (PO.sub.2) profile within individual spheroids at different stages of growth. Histological sections through the centers of spheroids were measured to determine the thickness of the viable rim of cells surrounding spheroid necrotic centers in order to estimate the size of the severely hypoxic zone of cells by comparison with the PO.sub.2 profiles of the same spheroids. The data demonstrate significant differences between these two human colon tumor spheroid systems. Both spheroid types exhibited steep PO.sub.2 gradients at relatively small sizes of <600-μm diameter, but for any given size in this range, the more differentiated Co112 spheroids were more hypoxic. Although severe hypoxia (PO.sub.2, <10 mm of Hg) was present in both spheroid types at larger sizes, there was a significant difference in the central PO.sub.2 values which were between 5 and 10 mm of Hg in large Co112 spheroids but remained at or close to 0 mm of Hg in large HT29 poorly differentiated human colon tumor spheroids. The presence of pseudoglandular structures and lumen in the Co112 spheroids was associated with changes in the shape of PO.sub.2 profiles. Such profiles have not previously been seen in other poorly differentiated human or rodent tumor spheroids. Furthermore, the PO.sub.2 profiles of both of these human tumor spheroid types were often continuously curving with a very shallow gradient in the inner edge of the viable rim of cells surrounding the necrotic center. Regulation of oxygen consumption and/or diffusion in these inner regions of human spheroids could produce these continuously curving PO.sub.2 gradients.

L7 ANSWER 27 OF 33 LIFESCI COPYRIGHT 2009 CSA on STN
ACCESSION NUMBER: 1999:40526 LIFESCI <<LOGINID::20090224>>
TITLE: Laser tweezers are sources of two-photon excitation
AUTHOR: Koenig, K.
CORPORATE SOURCE: Institute of Anatomy II, Friedrich Schiller University, D-07743 Jena, Germany
SOURCE: Cellular and Molecular Biology [Cell. Mol. Biol.], (19980700) vol. 44, no. 5, pp. 721-733. Special Issue: Modern Laser Microscopy..
ISSN: 0145-5680.
DOCUMENT TYPE: Journal
FILE SEGMENT: W3
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The most important application of continuous wave (cw) near infrared (NIR) microbeams in cellular and molecular biology are single-beam gradient force optical traps, also called "laser tweezers". Laser tweezers have been used for optical picoNewton force determination as well as for 3D cellular and intracellular micromanipulation, such as optical spermatozoa transportation in laser-assisted in vitro fertilization. Light intensities in the MW/cm super(2) range are necessary to confine motile spermatozoa in the optical trap. The enormous photon concentration in space and time results in non- resonant two-photon excitation of endogenous and exogenous absorbers with electronic transitions in the ultraviolet and visible spectral range. Trap- induced two-photon excitation of intracellular flurorophores can be used to study metabolism and vitality of motile cells without additional fluorescence excitation sources. Therefore, laser tweezers as sources of two-photon excitation may act as novel nonlinear tools in cell diagnostics. The far red/NIR trapping radiation, in particular <800 nm, may also excite endogenous absorbers such as NAD(P)H, flavins, porphyrins and cytochromes. Excitation of these cellular absorbers may result in oxidative stress via type I and type II photooxidation processes. Severe non-linear-induced cell damage in a variety of cells confined in <800 nm traps was found. Two-photon induced destructive effects are enhanced in multimode traps due to longitudinal mode-beating phenomena. Pulsed laser sources are not suitable for safe optical trapping of living cells. The use of single frequency long-wavelength NIR traps (800 nm-1200 nm) for vital cell handling is recommended.

L7 ANSWER 28 OF 33 BIOTECHDS COPYRIGHT 2009 THOMSON REUTERS on STN
ACCESSION NUMBER: 2003-07471 BIOTECHDS <<LOGINID::20090224>>
TITLE: Novel polypeptide encoded by nucleotide sequence derived from human erythropoietin gene with single nucleotide polymorphisms, for diagnosing, preventing and treating cancers, infections and autoimmune diseases;
vector-mediated recombinant protein gene transfer and expression in host cell for disease diagnosis and therapy
AUTHOR: ESCARY J
PATENT ASSIGNEE: GENODYSSEE
PATENT INFO: WO 2002085940 31 Oct 2002
APPLICATION INFO: WO 2002-EP4331 29 Mar 2002
PRIORITY INFO: US 2002-358598 21 Feb 2002; FR 2001-4603 4 Apr 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-093099 [08]
AN 2003-07471 BIOTECHDS <<LOGINID::20090224>>

AB DERWENT ABSTRACT:
NOVELTY - An isolated polypeptide (I) comprising all or part of a 193 residue amino acid sequence (S1), given in the specification, and having

at least one coding single nucleotide polymorphism (SNP) chosen from D70N, G104S and S147C, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an isolated polynucleotide (II) comprising all or part of a human wild-type erythropoietin (EPO) nucleotide sequence (S2) comprising 3398 base pairs, given in the specification, provided that such nucleotide sequence comprises at least one SNP chosen from 465-486 (deletion), c577t, g602c, c1288t, c1347t, g1644a, g2228a, g2357a, c2502t, c2621g, g2634a or its complement; (2) an isolated polynucleotide encoding (I); (3) genotyping (M1) all or part of a polynucleotide having 80-100 % identity with S2, by amplifying a region of interest in the genomic DNA of a subject or a population of subjects and determining the allele of at least one of the SNP positions as above in S2; (4) a recombinant vector (III) comprising (II); (5) a host cell (IV) comprising (III); (6) separating (I); (7) a polypeptide encoded by (II); (8) a hyperglycosylated analog (V) of the polypeptide comprising amino acids 28-193 of S1 and having SNP G104S; (9) an immunospecific antibody (VI) resulting obtained by immunizing an animal with polypeptide encoded by (II); (10) a therapeutic agent (VII) comprising one or more compounds chosen from (I)-(VI); (11) determining statistically relevant associations between at least one SNP in the EPO gene, and a disease or resistance to disease; (12) diagnosing or determining a prognosis of a disease or a resistance to a disease, by detecting at least one SNP in the EPO gene; (13) identifying a compound among one or more compounds to be tested having a biological activity substantially similar to or higher than the activity of G104S mutated EPO gene product; (14) a compound (VIII) identified by the method of (13); (15) molecules (IX) characterized by helices A, B, C and D having cellular proliferative functional characteristics at least equal to that of wild-type human EPO and capable of binding to an EPO receptor, having at least one alteration in the amino acid sequence of the helix B thus resulting in binding of EPO receptor with higher affinity than that of wild-type human EPO; (16) improving the cellular proliferative functionality of an EPO-like molecule having a portion corresponding to the helix B portion of wild-type EPO and capable of binding to EPO receptor; (17) a therapeutic compound comprising (IX); (18) improving the functionality of human wild-type EPO molecule having helices A, B, C and D; and (19) a compound (X) produced by the method of (18).

BIOTECHNOLOGY - Preparation: (I) is isolated by culturing (IV) in a culture medium and separating the polypeptide from the culture medium (claimed). Preferred Polynucleotide: (II) comprises nucleotides 615-2763 of S2, provided that the sequence contains at least one coding SNP chosen from g1644a, g2357a and c26221g. (II) is composed of at least 10 nucleotides. Preferred Polypeptide: (I) comprises amino acids 28-193 of S1 and has SNP G104S. Preferred Method: (M1) is carried out by minisequencing. Preferred Compound: In (X), an amino acid corresponding to glycine 104 of S1 is altered.

ACTIVITY - Cytostatic; Immunosuppressive; Cardiant; Anorectic; Virucide; Anti-HIV (human immunodeficiency virus); Dermatological; Nootropic; Neuroprotective; Antiparkinsonian; Neuroleptic; Antidepressant; Antiallergic; Antiasthmatic; Antipsoriatic; Antirheumatic; Antiarthritic; Antiinflammatory; Antiulcer; Vulnerary; Hepatotropic; Antianemic; Osteopathic.

MECHANISM OF ACTION - Stimulator of megakaryocytopoiesis; Protects neurons against cell death induced by ischemia. The capacity of G104S mutated EPO to stimulate erythroid colony formation was evaluated and compared to that of wild-type EPO. Human bone marrow cells from healthy individuals were collected and separated on a ficoll gradient. Nucleated cells were plated in semisolid methyl cellulose. Mutated or wild-type EPO ranging from 0.25-10 ng/ml was then added to the culture medium. After 10 days of culture,

erythroid colonies were counted. The data clearly demonstrated that G104S mutated EPO stimulated erythroid colony formation. In particular, stimulation of erythroid colony formation by G104S mutated EPO was 30-50 % higher than that measured with wild-type EPO.

USE - (II) is useful for identifying or amplifying all or part of a polynucleotide having 80-100 % identity with nucleotide sequence S2, by hybridizing (II) with the polynucleotide under appropriate hybridization conditions. (IV) is useful for identifying an agent among one or more compounds to be tested which activates or inhibits the activity of (I), or a compound whose activity is potentiated or inhibited by (I), by contacting the host cells with compounds to be tested, and determining the activating or inhibiting effect upon the activity of the polypeptide or the agent. Determining the presence or absence or level of expression of (II), or the concentration, functionality or presence or absence of polypeptide encoded by (II) is useful for analyzing the biological characteristics of a subject. (VII) or (VIII) is useful for preventing or treating in an individual a disease such as cancers, tumors, infectious diseases, venereal diseases, immunologically related diseases and/or autoimmune diseases and disorders, cardiovascular diseases, metabolic diseases, central nervous system diseases, gastrointestinal disorders, and disorders connected with chemotherapy treatments. Cancers and tumors comprise metastasizing renal carcinomas, melanomas, lymphomas comprising follicular lymphomas and cutaneous T cell lymphoma, leukemias comprising chronic lymphocytic leukemia and chronic myeloid leukemia, cancers of the liver, neck, head and kidneys, multiple myelomas, carcinoid tumors and tumors that appear following an immune deficiency comprising Kaposi's sarcoma in the case of AIDS. The metabolic diseases comprise non-immune associated diseases such as obesity. The infectious diseases comprise viral infections including chronic hepatitis B and C and human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS), infectious pneumonias, and venereal diseases such as genital warts. Diseases of the central nervous system comprise Alzheimer's disease, Parkinson's disease, schizophrenia and depression. Immunologically and auto-immunologically related diseases comprise the rejection of tissue or organ grafts, allergies, asthma, psoriasis, rheumatoid arthritis, multiple sclerosis, Crohn's disease and ulcerative colitis. Cardiovascular diseases include brain injury and anemias including anemia in patients under dialysis in renal insufficiency, as well as anemia resulting from chronic infections, inflammatory processes, radiotherapies and chemotherapies. (VII) or (VIII) is also useful for preventing or treating healing of wounds and/or osteoporosis in a individual and for increasing production of autologous blood, notably in patients participating in a differed autologous blood collection program. (I)-(VI) is useful for increasing or decreasing the activity of polypeptide encoded by (I) in a subject and for preventing or treating a disorder or disease linked to the presence of (I) in the genome of an individual. (IX) is useful for treating an individual. (All claimed.)

ADMINISTRATION - 1-300 units/kg of (I) is administered. Administration routes are not specified.

EXAMPLE - Three-dimensional structure of erythropoietin (EPO) was constructed starting from that available in the PDB database and by using the software modeler. The mature polypeptide fragment was modified to reproduce the mutation D70N, G104S or S147C. Polymerase chain reaction (PCR) amplification of the nucleotide sequence of the EPO gene was carried out starting from genomic DNA from 268 individuals of ethnically diverse origins. PCR amplification was carried out using specific primers designed on the basis of the nucleotide sequence of human wild-type EPO, comprising a sequence (S1) of 3398 base pairs given in the specification. For genotyping g1644a, PCR amplification was carried out using the primers: sense primer: TTCAGGGACCCTTGACTC and

antisense primer: GATCATTCTCCCTTTCATCC. These nucleotide sequences permit amplification of a fragment of a length of 208 nucleotides, from the nucleotide 1557-1764 in S1. For the genotyping of c2621g, PCR amplification was carried out using sense primer: TTGCATACCTTCTGTTTGCT and antisense primer: CACAAGCAATGTTGGTGAG. These sequences permitted amplification of a fragment of a length of 626 nucleotides, from the nucleotide 2192-2817 in S1. For each single nucleotide polymorphism (SNP) to be genotyped, the PCR product served as a template for the minisequencing. PCR product was purified by enzymatic digestion. Elongation or minisequencing step was then carried out on this digested PCR product by addition of a reaction mixture of 5 micro-l/prepared sample. For the SNP g1644a, the minisequencing primers tested were sense primer (A): tgcagcttgaatgagaatatcactgtccca; and antisense primer (B): cctcttccaggcatagaaattaactttggtgt. For the SNP c2621g, the minisequencing primers tested were sense primer (A): ttggcagaaggaagccatct, and antisense primer (B): ctgaggccgcatctggaggg. After the completion of genotyping process, the determination of the genotypes of the individuals of the population for the two functional SNPs was carried out. For the SNP g1644a, the genotype was in theory either homozygote GG, or heterozygote GA or homozygote AA in the tested individuals. In reality, the homozygote genotype GG was not detected in the population of individuals. Similarly, for the SNP c2621g, this genotype was in theory either homozygote CC or heterozygote CG, or homozygote GG in the tested individuals. In reality the homozygote genotype GG was not detected in the population of individuals. The distribution of determined genotypes in the population of individuals was determined. In the case of SNP g1644a, the only heterozygote individual GA came from the sub-population European Caucasoid of the population of individuals and in the case of SNP c2621g, the only heterozygote individual CG was from the sub-population European Caucasoid of the population of individuals. (76 pages)

L7 ANSWER 29 OF 33 BIOTECHDS COPYRIGHT 2009 THOMSON REUTERS on STN
ACCESSION NUMBER: 2003-02245 BIOTECHDS <<LOGINID::20090224>>
TITLE: A co-culture, useful as mammalian transplant tissue, comprises hepatocytes and hepatic stellate cells formed on a surface which is substantially free of molecules which provide signals to cells in the co-culture;
human cell culture, biodegradable material surface, growth factor and protein solution for artificial organ and tissue engineering
AUTHOR: SHAKESHEFF K; BHANDARI R N B; RICCALTON-BANKS L A; QUIRK R
PATENT ASSIGNEE: UNIV NOTTINGHAM
PATENT INFO: WO 2002048318 20 Jun 2002
APPLICATION INFO: WO 2001-GB5566 17 Dec 2001
PRIORITY INFO: GB 2000-30584 15 Dec 2000; GB 2000-30584 15 Dec 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-627259 [67]
AN 2003-02245 BIOTECHDS <<LOGINID::20090224>>
AB DERWENT ABSTRACT:
NOVELTY - A co-culture (I) comprising hepatocytes and hepatic stellate cells formed on a surface which is substantially free of molecules which provide signals to cells in the co-culture, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) culturing (M1) hepatocytes, by co-culturing the hepatocytes with hepatic stellate cell on a surface which is substantially free of molecules which provide signals to the cells in the co-culture; and (2) a surface (II) for (I), where (II) is substantially free of molecules which are capable of providing

signals to the cells in the co-culture.

BIOTECHNOLOGY - Preferred Co-culture: In (I), the surface comprises a polymeric material or a biodegradable material such as poly(alpha-hydroxy acid), preferably poly(lactic acid) or a copolymer of poly(lactic acid-co-glycolic acid). (I) is substantially free of added growth factors, extracellular matrix proteins or other cell types. The hepatocytes and/or the hepatic stellate cells are derived from human liver tissue. Preferred Method: In (M1), the surface is treated prior to seeding of the cells to block non-specific surface interactions. The surface is treated with a protein solution, preferably a bovine serum albumin solution.

USE - (I) is useful in in vitro toxicology testing of substances or metabolism testing of substances, where the substances are drugs or environmental pollutants, as mammalian transplant tissue, preferably human transplant tissue, as a component of engineered liver tissue for implantation into a human, or as a component of a liver-assist device (claimed).

ADVANTAGE - (I) has improved functionality in terms of their ability to metabolize drugs using cytochrome P450 enzymes.

EXAMPLE - Fresh rat hepatocytes were isolated and purified using a two-step collagenase perfusion method. Liver lobes were removed from male Wistar rats and perfused first with a calcium-free 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) buffer and then with calcium- and collagenase- containing HEPES buffer. The lobes were then minced and filtered through cotton gauze to release the hepatocytes. The resultant cell suspension was washed twice by centrifugation. Hepatocytes, suspended in Williams E medium (WEM) containing 10% fetal calf serum (FCS) were separated from non-viable and non-parenchymal cells using a percoll density gradient centrifugation. The cells were then resuspended in WEM with FCS and used the same day. Viability, as determined using the trypan blue exclusion method, was between 85-95%. Stellate cells were collected from the hepatocyte isolation by pooling the collagenase perfusate and washings, which were then stored. The suspension was then aliquotted into 50 ml centrifuge tubes and spun at 600 rpm for 1 minute at 4 degrees Centigrade . After discarding the pellet, the supernatant was again spun at 600 rpm, this time for 3 minutes at 4 degrees Centigrade . This procedure was repeated three times, and the final centrifugation was performed at 900 rpm for 5 minutes. After the third spin, the supernatant was discarded and the pellet was resuspended in 10 ml phosphate buffered saline (PBS), which was then spun twice at 900 rpm for 5 minutes at 4 degrees Centigrade . The cells remaining in the pellet were finally resuspended in 20 ml Dulbecco's modified Eagle medium (DMEM) containing 10% FCS and then seeded in a culture flask. The media was changed after 5 hours. Cell attachment and co-culture was performed on modified poly (D,L-lactic acid) (PLA) surfaces. Stellate cells were incubated with Cell Tracker (RTM) green 5-chloromethylfluorescein diacetate (CMFDA) fluorescent stain by reconstituting the material in 11 microl dimethyl sulfoxide (DMSO) and adding this solution to the media of a confluent tissue culture flask for 45 minutes. The media was then replaced and the cells were incubated for a further hour. Following trypsinization and resuspension, these cells were centrifuged and resuspended in serum-free DMEM. Isolated hepatocytes were centrifuged and resuspended in 9 serum-free WEM. Co-culture studies used a suspension containing 20000 cells/ml of both hepatocytes and stellates. After 5 hour incubation, unadhered cells were removed by washing the PLA discs with fresh media three times. Cell attachment was investigated using a combined light and fluorescence microscopy approach in order to distinguish between the two cell populations. 10 images were taken from duplicate wells after a 5 hour

incubation period. In all experiments, cells were grown simultaneously on tissue culture plastic in serum-containing medium as a positive control. The effect of surface on co-cultures was monitored over a five-day period and within 3 hours of seeding stellate cells began to spread on the PLA surface, an event that did not occur in a single-cell type environment. By the second day, these cells were sending out processes specifically towards hepatocytes and on day three, most cells had clustered together to form multilayered spheroid-type structures. These cell structures mimicked the morphology of in vivo liver lobules and exhibited enhanced activity and prolonged functionality compared with hepatocyte monolayers. (21 pages)

L7 ANSWER 30 OF 33 BIOTECHDS COPYRIGHT 2009 THOMSON REUTERS on STN
ACCESSION NUMBER: 2003-01461 BIOTECHDS <<LOGINID::20090224>>
TITLE: Two acyl-CoA dehydrogenases of Acinetobacter sp strain M-1 that uses very long-chain n-alkanes;
recombinant enzyme production and purification via vector expression in host cell and polymerase chain reaction
AUTHOR: TANI A; ISHIGE T; SAKAI Y; KATO N
CORPORATE SOURCE: Kyoto Univ
LOCATION: Kato N, Kyoto Univ, Grad Sch Agr, Div Appl Life Sci, Sakyo Ku, Kyoto 6068502, Japan
SOURCE: JOURNAL OF BIOSCIENCE AND BIOENGINEERING; (2002) 94, 4, 326-329
ISSN: 1389-1723
DOCUMENT TYPE: Journal
LANGUAGE: English
AN 2003-01461 BIOTECHDS <<LOGINID::20090224>>
AB AUTHOR ABSTRACT - Two genes encoding acyl-CoA dehydrogenases, acdA and acdB, arranged in tandem, were found in the chromosomal DNA of Acinetobacter sp. strain M-1. AcdAwas purified from the parental strain and AcdB was purified from an Escherichia coli strain expressing the cloned gene. The substrate specificities of the two enzymes suggest that AcdA is a medium-chain acyl-CoA dehydrogenase and that AcdB is a long-chain acyl-CoA dehydrogenase. Characterization ofn-alkane metabolism in Acinetobacter sp. strain M-1 has revealed parallelpathways as well as enzymes with overlapping specificities in a single pathway. The two acyl-CoA dehydrogenases described here provide another exampleof the physiological complexity underlying n-alkane utilization.
DERWENT ABSTRACT: Acyl-CoA dehydrogenase, designated AcdA, was purified through the following steps at 4 deg. The soluble fraction of cell-free extract of Acinetobacter sp. strain M-1 in the late logarithmic phase was chromatographed on a DEAE-Toyopearl column (2.5 x 20 cm) preequilibrated with 50 mM Tris-HCl buffer, pH 8.5 (buffer-A). Elution was performed with a linear gradient of increasing NaCl concentration (0 M-1 M in a total elution volume of 1600 ml). The active fractions, to which ammonium sulfate was added to a concentration of 1.2 M, were applied to a Butyl-Toyopearl column (2.5 x 20 cm) preequilibrated with buffer-A containing 1.2 M ammonium sulfate. The enzyme was eluted with a linear gradient of decreasing ammonium sulfate concentration (1.2 M-0 M, in a total volume of 1000 ml). After concentration by ultrafiltration using a Diaflow membrane YM30 and gel filtration on a Superdex 200 column (1.6 x60 cm, equilibrated with buffer-A containing 0. 1 M NaCl), the enzyme was applied to a Q-Sepharose column (1.8 x 10 cm) preequilibrated with buffer-A (total volume, 400 ml). The active fractions were collected and used as the purified enzyme(4 pages)

L7 ANSWER 31 OF 33 DISSABS COPYRIGHT (C) 2009 ProQuest Information and

ACCESSION NUMBER: 87:22027 DISSABS Order Number: AAR8727956
TITLE: HIGHER-ORDER CELL SURFACE COMPLEXES ON SELECTED METASTATIC MELANOMA CELL LINES: ANALYSES USING ONE AND TWO DIMENSION CHAPS/SDS POLYACRYLAMIDE GRADIENT GEL ELECTROPHORESIS
AUTHOR: UPDYKE, TIMOTHY VALE [PH.D.]
CORPORATE SOURCE: UNIVERSITY OF CALIFORNIA, IRVINE (0030)
SOURCE: Dissertation Abstracts International, (1987) Vol. 48, No. 10B, p. 2964. Order No.: AAR8727956. 139 pages.
DOCUMENT TYPE: Dissertation
FILE SEGMENT: DAI
LANGUAGE: English
ENTRY DATE: Entered STN: 19921118
Last Updated on STN: 19921118

AB Extensive efforts made by metastasis investigators to determine the biochemical mechanisms and cell-surface molecular associations that participate in the various cellular interactions occurring before, during, or after the highly selective steps of the metastatic process have yielded primarily subtle quantitative differences, if any, between cell lines of diverse metastatic potential. One reason for such results may be that most biochemical analyses of cell-surface molecules utilize highly reductionistic methodologies that include strong denaturants, such as sodium dodecyl sulfate (SDS) or urea, that yield well resolved subunits, with little, if any, of the structural and functional organization of cell-surface components left intact. The differential cell-surface organization and topology of otherwise identically expressed components may be important in determining the functional differences between cell populations. In this dissertation a native polyacrylamide gradient gel electrophoresis (PAGGE) system is described that was developed to study the native structure and organization of the cell-surface components of metastatic B16 melanoma cell lines that were selected in vitro for increased homotypic adhesion properties and that displayed an increased ability to form tumor colonies in the lungs of syngeneic mice when compared to the parental cell line.

The proteins and glycoproteins of the cell lines were labeled with various metabolic and cell-surface labeling agents both before and after the cells were allowed to undergo homotypic aggregation. The cells were solubilized in buffers that contained the zwitterionic detergent 3-(3-cholamidopropyl)-dimethylammonio-1-propanesulfonic acid (CHAPS). The CHAPS-lysates were applied to the native-gels that contained CHAPS, a high resolution moving boundary electrophoresis buffer system, and continuous, linear polyacrylamide gradients. With CHAPS-PAGGE differences were seen in several higher-order as well as low molecular size components both between the cell lines and between single and aggregated cells. None of the differences seen with CHAPS-PAGGE were seen with SDS-PAGGE; however, when the components in the CHAPS-PAGG were run through a second dimension SDS-PAGG (Native-Dalt) further differences were observed between the cell lines, between single and aggregated cells, and between samples that were run with and without ethylenediaminetetraacidic acid (EDTA). The results with the CHAPS-PAGGE and Native Dalt systems demonstrate that it should now be possible to identify important cell-surface components on cells and how they associate during cellular interactions.

L7 ANSWER 32 OF 33 DISSABS
ACCESSION NUMBER: 87:6976 DISSABS Order Number: AAR0560311 (not available for sale by UMI)
TITLE: CLOFAZIMINE-MEDIATED INHIBITION OF HUMAN POLYMORPHONUCLEAR

LEUCOCYTE MIGRATION: A NOVEL PRO-OXIDATIVE MECHANISM OF ANTI-INFLAMMATORY ACTIVITY. (AFRIKAANS TEXT)
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CORPORATE SOURCE: UNIVERSITY OF PRETORIA (SOUTH AFRICA) (6004)
SOURCE: Dissertation Abstracts International, (1987) Vol. 48, No. 3B, p. 646. Order No.: AAR0560311 (not available for sale by UMI).
DOCUMENT TYPE: Dissertation
FILE SEGMENT: DAI
LANGUAGE: English
ENTRY DATE: Entered STN: 19921118
Last Updated on STN: 19921118

AB Preliminary studies on the in vitro and in vivo effects of clofazimine on the function of polymorphonuclear leucocytes (PMNL) from normal individuals and patients with lepromatous leprosy showed that clofazimine caused a progressive dose-dependent inhibition of both random motility of PMNL as well as migration of PMNL induced by the leucoattractant endotoxin-activated serum (EAS). The drug also increased chemiluminescence as well as hexose monophosphate shunt (HMS) activity. After ingestion of 200 mg of clofazimine daily for a period of five days random migration and EAS-induced migration in normal individuals were decreased. These results were later confirmed and extended in mechanistic studies using an improved method of solubilisation of clofazimine.

The effects of clofazimine in vitro on migration, membrane-associated oxidative metabolism, degranulation, microtubule assembly and production of prostaglandin (PG) E(,2) by human PMNL of normal individuals were investigated in further studies. Clofazimine increased spontaneous and N-formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP)-stimulated chemiluminescence, hexose monophosphate shunt (HMS) activity, auto-iodination, degranulation, microtubule assembly and PGE(,2) production. Inhibition by clofazimine of random migration as well as chemotaxis, chemokinesis and orientation in a FMLP-gradient was observed. This inhibition of PMNL motility was prevented by the anti-oxidants cysteine and dapsone but not by the PG synthetase inhibitors indomethacin and piroxicam. Clofazimine also caused a functional inactivation of FMLP which could be prevented by co-incubation with an anti-oxidant. These results show that inhibition of PMNL migration by clofazimine was due to a cell-directed auto-oxidative mechanism as well as functional oxidative inactivation of FMLP.

Clofaximine is not an oxidising agent nor does it stimulate membrane-associated oxidative metabolism in PMNL of patients with chronic granulomatous disease or sodium fluoride-pulsed PMNL of normal individuals. Clofazimine-mediated inhibition of PMNL migration is therefore dependent on intact membrane-associated oxidative metabolism. Clofazimine is therefore a pro-oxidative anti-inflammatory agent.

L7 ANSWER 33 OF 33 DISSABS
ACCESSION NUMBER: 86:18337 DISSABS Order Number: AAR8626517
TITLE: TRANSFERRIN AND TRANSFERRIN RECEPTORS: REGULATION AND IMMUNE FUNCTION
AUTHOR: DIRUSSO, STEPHEN MICHAEL CHRISTOPHER [PH.D.]
CORPORATE SOURCE: EMORY UNIVERSITY (0665)
SOURCE: Dissertation Abstracts International, (1986) Vol. 47, No. 8B, p. 3286. Order No.: AAR8626517. 200 pages.
DOCUMENT TYPE: Dissertation
FILE SEGMENT: DAI
LANGUAGE: English
ENTRY DATE: Entered STN: 19921118
Last Updated on STN: 19921118

AB Transferrin is the major iron binding protein in serum and is

necessary for the proliferation of all studied mammalian cells. It plays a central role in the regulation of cell growth and interacts with cells via a specific cell surface receptor. This thesis investigates the distribution of iron on human transferrin and studies several physiological consequences of abnormal concentrations of human transferrin. First, using a novel polyacrylamide gradient gel electrophoretic method to quantitate apo-, mono-, and diferric transferrin based on differences in their molecular size, I showed that the saturation of iron on transferrin of normal individuals was random, and that certain patients with disorders of iron and/or transferrin metabolism had different distributions. Second, I utilized an in vivo model, the nephrotic syndrome of childhood, to show that hypotransferrinemia can account for some of the demonstrated abnormalities in humoral and cellular immune function seen in these patients. Third, using flow cytometry and monoclonal antibodies, I demonstrated that changes in transferrin concentration regulates transferrin receptor density. Three human cell lines of T (KT), B (Raji), and erythropoietic (K562) origin, as well as mitogen stimulated human peripheral blood mononuclear cells were subjected to acute or chronic deprivation of serum or transferrin. Increased expression of transferrin receptors in the cell lines grown in transferrin deficient medium was observed and was shown to be due to a specific increase in transferrin receptor synthesis. The physiologic implications of increased receptor density included an enhanced ability to endocytose transferrin and a close relationship between the ability of cells to increase receptors and the growth phase of the cell cultures. The increase in transferrin receptor density observed with malignant cell lines did not extend to normal blood mononuclear cells. These data do not rule out and in vivo role for transferrin concentration in the regulation of receptor expression in normal cells. Nevertheless, since the increase in transferrin receptor density allows transformed cells to grow in low transferrin in vivo, this mechanism might also provide a selective advantage for malignant cells over normal cells in vivo.

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